1. Introduction to PEAKS 5.1 ................................................................................6
   1.1 Main Features.............................................................................................. 6
   1.2 New Features in the PEAKS 5 Product Line .............................................. 6
   1.3 Workflow .................................................................................................... 7
   1.4 Guidelines for Using this Manual ............................................................... 7
   1.5 Scope ......................................................................................................... 7
   1.6 Service and Support .................................................................................. 7
2. Getting Started with PEAKS 5.1 ....................................................................8
   2.1 Package Contents ....................................................................................... 8
   2.2 System Requirements .............................................................................. 8
      2.2.1 Adjusting the Amount of Memory that PEAKS Uses .................... 8
   2.3 Installation for Windows Users ................................................................. 9
   2.4 Registering PEAKS .................................................................................. 10
      2.4.1 Registration Instructions (Internet Connection) ............................... 10
      2.4.2 Registration Instructions (No Internet Connection) ....................... 11
      2.4.3 Re-registration Instructions .............................................................. 14
   2.5 Set up PEAKS Preferences ..................................................................... 14
   2.6 Set up PEAKS Configuration .................................................................. 14
3. Quick Walkthrough .................................................................................. 15
   3.1 Create a Sample Database ........................................................................ 15
   3.2 Create a Project ....................................................................................... 15
   3.3 Perform Data Refinement ........................................................................ 17
   3.4 Run de novo Sequencing ......................................................................... 18
   3.5 Run Protein Identification ...................................................................... 21
   3.6 Run PTM Finder ...................................................................................... 23
   3.7 Run an inChorus Search ......................................................................... 25
   3.8 Perform a SPIDER Search ...................................................................... 30
4. Load Data .................................................................................................... 33
   4.1 Data Formats ............................................................................................ 33
   4.2 Data Conversion ....................................................................................... 33
      4.2.1 PEAKS Project Converter ............................................................... 33
      4.2.2 Agilent Data ...................................................................................... 34
      4.2.3 Applied Biosystems (WIFF)Data ...................................................... 34
      4.2.4 Applied Biosystems (4700/4800)Data ............................................ 35
      4.2.5 Bruker Data ...................................................................................... 35
      4.2.6 Shimadzu Data ............................................................................... 35
      4.2.7 Thermo Data .................................................................................... 36
      4.2.8 Varian Data ..................................................................................... 36
      4.2.9 Waters/Micromass (MassLynx) Data ............................................. 36
   4.3 Create a New Project ............................................................................... 37
   4.4 Add Data to an Existing Project .............................................................. 38
   4.5 Open / Close a Project .......................................................................... 39
   4.6 Delete and Hide Results ......................................................................... 39
   4.7 Change the Location of Saved Projects ............................................... 39
   4.8 Orienting Yourself .................................................................................. 40
4.8.1 Project View Panel ................................................................. 40
4.8.2 Properties Panel ................................................................. 40
4.8.3 Raw Spectrum View ........................................................... 41

5. Data Refinement ........................................................................ 43
  5.1 Run Data Refine ..................................................................... 43
  5.2 Data Refinement Parameters .................................................. 44
    5.2.1 Merging Scans ............................................................... 44
    5.2.2 Precursor Charge Correction ........................................ 44
    5.2.3 Filtering MS/MS Scans .................................................... 44
    5.2.4 Preprocessing MS/MS Scans .......................................... 44
  5.3 Data Preprocessing Results .................................................... 44

6. \textit{de novo} Sequencing ............................................................ 45
  6.1 Setting up Auto \textit{de novo} Sequencing Parameters .................. 45
    6.1.1 Mass Options .............................................................. 46
    6.1.2 Enzyme Options .......................................................... 46
    6.1.3 PTM Options ............................................................... 46
    6.1.4 General Options .......................................................... 46
  6.2 \textit{de novo} Sequencing Results ............................................ 47
    6.2.1 Peptide Candidates Frame .............................................. 48
    6.2.2 Ion Table Frame ........................................................... 50
    6.2.3 Spectrum View Frame ................................................... 50
    6.2.4 Spectrum Alignment Frame .......................................... 51
    6.2.5 Survey Scan ................................................................. 51
    6.2.6 Error Map ................................................................. 51
  6.3 Manual \textit{de novo} Sequencing ............................................. 51
    6.3.1 Manual \textit{de novo} Graphical User Interface ..................... 51
    6.3.2 Manual \textit{de novo} Operations ....................................... 53

7. Database Search ......................................................................... 58
  7.1 Setting up Protein Identification Parameters ............................. 58
    7.1.1 Mass Options .............................................................. 59
    7.1.2 Enzyme Options .......................................................... 59
    7.1.3 General Options .......................................................... 59
    7.1.4 PTM Options ............................................................... 60
    7.1.5 Database Options ........................................................ 61
    7.1.6 Advanced Options ....................................................... 61
  7.2 Protein Identification Results .................................................. 62
    7.2.1 Peptide View ............................................................... 62
    7.2.2 Protein View .............................................................. 64

8. SPIDER Search .......................................................................... 69
  8.1 Setting up SPIDER Parameters ............................................. 69
    8.1.1 Query Options ............................................................ 70
    8.1.2 General Options .......................................................... 70
    8.1.3 PTM Options ............................................................... 71
    8.1.4 Filter Options .............................................................. 71
    8.1.5 \textit{de novo} Options ....................................................... 72
    8.1.6 Database Options ........................................................ 72
8.2 SPIDER Results View........................................................................................................ 72
9. PTM Finder ........................................................................................................................ 74
  9.1 Setting up PTM Finder Parameters........................................................................... 74
  9.2 PTM Finder Results View....................................................................................... 75
10. inChorus Meta Search.................................................................................................. 76
  10.1 Setting up inChorus Parameters .......................................................................... 76
    10.1.1 Importing Existing Results .................................................................... 77
  10.2 inChorus Results View ....................................................................................... 78
    10.2.1 de novo, Peptide and Protein Views....................................................... 78
11. Creating a High-Throughput Work Flow .................................................................... 79
  11.1 Setting up the Work Flow Parameters................................................................. 79
12. Filtering Your Results .................................................................................................... 81
  12.1 Setting Filter Parameters ...................................................................................... 81
    12.1.1 Possible Filters/ Selected Filters/ Edit Filter ........................................ 82
    12.1.2 Filter Options .......................................................................................... 83
    12.1.3 Parameter Options.................................................................................. 84
13. Complex Analysis.......................................................................................................... 86
  13.1 Creating a project for complex system ................................................................. 86
  13.2 Integrating data analysis........................................................................................ 86
14. Exporting Data/Reports and Printing.......................................................................... 87
  14.1 Export Data in .mzxml or .mgf.............................................................................. 87
  14.2 Export Results File.................................................................................................. 87
  14.3 Export Peptide Results in PepXML Format ........................................................ 88
  14.4 Export Results in Excel Format............................................................................. 88
  14.5 Print Tables and Graphs for Publication ............................................................. 89
    14.5.1 de novo Image Files ................................................................................. 89
    14.5.2 Protein ID Image Files............................................................................. 90
15. Advanced Configuration and Environment Preferences............................................ 92
  15.1 PEAKS Environment Preferences......................................................................... 92
    15.1.1 General Preferences................................................................................ 92
    15.1.2 Instrument Preferences .......................................................................... 94
    15.1.3 Search Engine Preferences..................................................................... 96
    15.1.4 Ion Editor Preferences............................................................................ 98
  15.2 PEAKS Configuration............................................................................................ 99
    15.2.1 Enzyme Configuration............................................................................. 99
    15.2.2 PTM Configuration .............................................................................. 100
    15.2.3 Database Configuration......................................................................... 101
    15.2.4 Instrument Configuration.................................................................... 105
    15.2.5 Parameter Configuration..................................................................... 107
16. PEAKS Quantification Module .................................................................................. 108
  16.1 Setting up PEAKS Q Parameters........................................................................... 108
  16.2 3D View................................................................................................................. 110
  16.3 iTRAQ Walkthrough............................................................................................ 111
    16.3.1 Setting up a Database............................................................................. 111
    16.3.2 Creating a Project.................................................................................. 112
1. Introduction to PEAKS 5.1

1.1 Main Features
PEAKS is an innovative software system designed to derive amino acid sequences and identify proteins using tandem mass spectrometry data from all major mass spectrometry vendors. PEAKS incorporates *de novo* sequencing results into the database searching process for peptide/protein identification. It does this by generating sequence tags which are used in conjunction with fragment ion mass matching to speed up the search, remove false positive matches, and find peptides with interesting sequence variations or modifications that would prevent them from being otherwise identified. Our meta protein search tool, inChorus allows users to use multiple search engines (PEAKS, X!Tandem, OMSSA Sequest and Mascot) to expand sequence coverage and increase confidence. Another feature is SPIDER, which is a homology search tool for *de novo* sequencing. SPIDER is also able to reconstruct the correct sequence using the *de novo* sequence and a homologous peptide.

1.2 New Features in the PEAKS 5 Product Line
We have many new features in PEAKS 5.1 which will be explained throughout this manual. One main feature which required a complete redesign of the program architecture is the ability to handle very large data sets. In addition, our protein identification is more sensitive and generates less false positives.

BSI, creator of PEAKS, have also produced a quantification module that will allow users to automatically quantify proteins from experiments using both label and label-free techniques. Results generated from PEAKS Q have high accuracy and can be performed over a wide dynamic range with very large datasets. PEAKS 5.1 also has improved identification of PTMs with our innovative PTM finder.

PEAKS 5.1 uses project based data management which allows users to process simultaneous runs and easily compare/contrast samples within one project.
1.3 Workflow

1.4 Guidelines for Using this Manual
This user's manual is intended to help you get started with PEAKS 5.1. It will describe its functionalities, show how to customize PEAKS to your applications, provide a task based reference, and troubleshooting. We recommend reading the walkthrough in Chapter 3, using the sample data provided.

1.5 Scope
PEAKS users are assumed to be familiar with computer usage and the operating system environment. As such, it is beyond the scope of this manual to instruct the user on the use of windows, dialogue boxes, menus, file storage etc. Please refer to the operating system’s manual or computer help books for such information. Similarly, PEAKS users are expected to be familiar with mass spectrometry, standard operating practices and data.

1.6 Service and Support
In addition to reading this manual, we recommend that you take a look at our training videos that explain the main features of PEAKS in detail (http://www.bioinfor.com/products/peaks/support/tutorials.php). Please send technical questions to support@bioinfor.com.

We also encourage our users to provide us with any suggestions or comments as we are always improving PEAKS to meet the needs of the scientific community (http://www.bioinformaticssolutions.com/corporate/contactform.php).
2. Getting Started with PEAKS 5.1
This section of the manual will guide you through the installation and configuration of PEAKS 5.1.

2.1 Package Contents
The PEAKS 5.1 package contains:
- This manual (hardcopy and/or electronic version)
- PEAKS 5.1 software

2.2 System Requirements
PEAKS 5.1 will run on most platforms with the following minimum requirements:
- Processor: Equivalent or superior processing power to an Intel® Pentium 4 Processor 1.6GHz (we recommend an Intel® Core™2 Duo processor/AMD Phenom™ processor)
- Memory: 1 GB memory (we recommend 2GB memory)
- Operating System: PEAKS runs on Windows XP and Vista.

2.2.1 Adjusting the Amount of Memory that PEAKS Uses
The PEAKS Studio directory (e.g., C:/PEAKS Studio 5.1) contains a file called Memory Utility.exe. Click to open this file and the following window will open:

The default of 1024 MB tells the Java Virtual Memory (which runs PEAKS) to run with 1024 MB of memory. You can increase the Java VM to determine the highest optimal value for your own computer although some trial and error may be needed as Java will not start if you set the value to be too large for your system. Please do not hesitate to contact us if you have any questions about your own system.
2.3 Installation for Windows Users

Note: If you already have an older version of PEAKS installed on your system, please uninstall it before proceeding.

1. Close all programs that are currently running.

2. Insert the PEAKS 5.1 disc into the CD-ROM drive. If loading PEAKS via the download link, skip to step 4, after downloading and running the file.

3. Auto-run should automatically load the installation software. If it does not, find the CD-ROM drive and open it to access the disc. Click on the PEAKS_Studio_Installation.exe.

4. A menu screen will appear. Select the top item “PEAKS Installer.” The installation utility will begin the install. Wait while it does so. When the “PEAKS 5.1” installation dialogue appears, click the “Next” button.

5. Basic system requirements will be presented. Please note that while the minimum requirement is 1 GB of RAM, the preferred configuration is 1.5 GB of RAM. “Click Next”.

6. Read the license agreement. If you agree with it, change the radio button at the bottom to select “I accept the terms of the License Agreement” and click “Next”.

7. Choose the folder/directory in which you would like to install PEAKS. The default location is simply “C:\PeaksStudio5.1. To change this location press the “Choose…” button to browse your system and make a selection, or type a folder name in the textbox. Please avoid installing PEAKS in the “Program Files” directory as well as in any directory for which the PEAKS user will not have write-permissions. Click “Next”.

8. Choose where you would like to place icons for PEAKS 5.1. The default will put these icons in the programs section of your start menu. A common user preference is on the desktop. Click “Next”.

9. Review the choices you have made. You can click “Previous” if you would like to make any changes or click “Next” if those choices are correct.

10. PEAKS 5.1 will now install on your system. You may cancel at any time by pressing the “Cancel” button in the lower left corner.
11. When the installation is complete, click “Done”. The PEAKS 5.1 menu screen should still be open. You may view movies and materials from here. To access this menu at a future date, simply insert the disc in your CD-ROM drive.

2.4 Registering PEAKS

The first time PEAKS is run, the license wizard will appear automatically:

2.4.1 Registration Instructions (Internet Connection)
1. Select “Request license file (has Internet connection)” and click “Next”.
2. The following window will appear:

   ![License Wizard]

If you have purchased PEAKS and have a registration key, select “Registration Key”. Enter your registration key as well as your name and email address and click “Next”.

OR

If you are trying a demo of PEAKS and do not have a registration key, select “Request a 30 days evaluation license (No registration key required). Enter your name, email address, as well as your institution. Click “Next”.

3. The following window will appear:

   ![License Wizard]

An automated BSI service will generate the license file (license.lcs) and email it to the provided email account from the License Wizard. You can either save the attachment to a local directory or copy the content between ‘===>’ and ‘<===’ in the email. Click “Next”.

   ![License Wizard]

The software registration request has been sent to BSI successfully. Please check your email to get the license file and use the “Import License” function to activate the software.
4. The following window will appear:

Select “Paste the license and click on the “Paste from Clipboard” button to paste the license information between ‘===>’ and '<===' in the email or select “Import the license file (the email attachment) and browse to locate the license file (license.lcs). Click “Next”.

5. The following window will open:

Click “Finish” if you receive a message that the license has been imported successfully.

6. Restart PEAKS to begin using the software.

2.4.2 Registration Instructions (No Internet Connection)

1. Select “Request license file (without Internet connection)” and click “Next” twice.
2. The following window will open:
If you have purchased PEAKS and have a registration key, select “Registration Key”. Enter your registration key as well as your name and email address and click “Next”.

OR
If you are trying a demo of PEAKS and do not have a registration key, select “Request a 30 days evaluation license (No registration key required)”. Enter your name, email address, as well as your institution. Click “Next”.

3. The following window will appear:
Select the “Save Request File” button to save license.request to your computer (PC1). Click “Next”.

4. Transfer the license.request file from PC1 onto a computer with an Internet connection (PC2) using a USB key or a removable storage device. On PC2, go to http://www.bioinfor.com/lcs20/

5. Select “I have the license request file. I want to register the software” and click “Next”.
6. The following window will appear:

Click the “Browse” button to select the license.request file, type in the visual verification code and click “Next”.

7. After the license email is received on PC2, save the attachment, license.lcs, as is and copy the file to PC1. If you do not receive the license.lcs file in your inbox, please check your junk mail folder.

8. In the license wizard on PC1, click the 'Browse' button below to select the license.lcs file and click “Next”.

9. Click “Finish” if you receive a message that the license has been imported successfully.

10. Restart PEAKS to begin using the software.
2.4.3 Re-registration Instructions

Re-registering PEAKS may be necessary if your license has expired or if you wish to update the license. You will need to obtain a new registration key from BSI. Once you have obtained this new key, select “About PEAKS Studio” from the Help menu. The “About BSI PEAKS Studio” dialogue box will appear:

Click the “License Wizard” button to continue. Then follow the instructions listed above for registering PEAKS.

2.5 Set up PEAKS Preferences

Before running your data, you must set up search engine preferences. For an explanation on how to do this, see section 15.1.3 Search Engine Preferences.

It is possible to run your data through PEAKS without changing the other preferences as they have default settings. For more information on changing these default settings see section 15.1 PEAKS Environment Preferences.

2.6 Set up PEAKS Configuration

Before running your data, you must configure your databases. For instructions on how to do this, see section 15.2.3 Database Configuration.

It is possible to run your data through PEAKS without configuring any other parameters as they have default settings. For more information on changing these default settings see section 15.2 PEAKS Configuration.
3. Quick Walkthrough

This section of the manual will walk you through most of the basic functionality of PEAKS 5.1. After completing this section you will see how easy it is to load, view a data file, perform data refinement, perform *de novo* sequencing, and database search protein identification.

Please note that version upgrades of PEAKS as well as upgrades to the databases may result in small changes to the results screenshots in this chapter.

3.1 Create a Sample Database

Before running the walkthrough data, you need to set up a database. So that this can be a quick process, we have provided you with a sample FASTA database called “SampleDB.fasta” in your PEAKS program folder (C:\PEAKSStudio5.1\Data).

Click on the configuration toolbar icon or select “Configuration” from the “Tools” menu. Select “Database” from the left hand side of the window. Under “Database Details” enter the following information:

You do not need to change any of the other information listed. Click the “Add/Update” button and then click “OK”.

3.2 Create a Project

This will be a rather simple project as it will only contain one sample; however the same process will be used for projects with multiple samples and files. Click on the “Create new project” button or select “New Project” from the “File” menu. The following window will appear:
Enter a name for your project. Do not change the default project location. By default the first sample will be named “Sample 1”. Click the “Add data files” button to add a data file for sample 1. Select the “OrbiSample.mzxml” file from your PEAKS program folder (for example “C:\PEAKSSStudio5.1\Data”) and click “Open”. Leave the instrument vendor as “All Instruments” and select “FT-trap” from the “Instrument Type” drop-down list.

Click “OK”.

You should now see the following in the “Project View” panel, which is located in the upper left hand corner of the screen:

Use the ‘+’ and ‘-’ boxes to expand and collapse the project in order to access the data file in the “Project View”. Make sure that you select this data file when choosing data to be analyzed.
PEAKS reads and tracks information about the experiment and data for use in the analysis and for future reference. Once the data file has loaded, the fraction icon will turn solid. Click on the “Properties” tab in the bottom left hand corner:

You should see the following in the “Main Processing Window”:

The information that is displayed by default pertains to the precursor scan. To the left of the window is the “Total Ion Chromatogram” (TIC). The graph in the upper right corner displays a survey scan with its corresponding tandem scans below. Click on the MS/MS tab to see the graphs that were generated from the tandem scan. For more information on the functions and tools found in these windows, see section 4.8 Orienting Yourself.

### 3.3 Perform Data Refinement

1) Click the Data Refine toolbar icon

   Or

   Select “Data Refine” from the “Tools” menu.

2) Enter the settings as shown:
For more details on setting up the parameters for data refinement refer to 5.2 Data Refinement Parameters.

Here we will use a quality filter to remove data with a quality value lower than 0.65. As all of the data in this data set is of good quality, we will not end up removing any data using this filter.

After running data refine, there will be new information listed in the “Properties” file.

### 3.4 Run de novo Sequencing

1) Click the *de novo* sequencing toolbar icon 📑

Or

Select “*de novo*” from the “Tools” menu.

2) Enter the settings as shown:
Enter the PTM as shown below by selecting “Set PTM”:

Note that we are not going to preprocess this data “on the fly” as we have already preprocessed the data during the data refinement stage. We will also choose to report only one peptide per spectrum for simplicity’s sake.

You can save the parameters that you used for future reference by clicking “Save As”. For more information on setting up de novo parameters see section 6.1 Setting up Auto de novo Sequencing Parameters. Click “OK” to commence analysis. The PEAKS auto de novo algorithm
derives sequence candidates for each of the six spectra in our example data file. Once the *de novo* sequencing is completed the results file will appear in the “Project View Panel”:

Double click on the *de novo* file and the results will appear in the “Main Processing Window”:

Take a look at spectra ID 6. Notice that the [1] refers to the modification which in this case is Carboxymethyl.

At the top of the screen you will see the peptide candidates in the “Peptide Candidates Frame”. The peptide candidates are sorted by “ID”. Right next to the proposed sequence, the auto *de*
“Total Local Confidence” (TLC) and “Average Local Confidence” (ALC%) confidence scores are shown. You will also see the \( m/z \) ratio, mass, retention time etc. listed for each peptide sequence. For information on the “Peptide Candidates Frame including color coding, see section 6.2.1 Peptide Candidates Frame.

Below the “Peptide Candidates Frame” is the “Ion Table Frame”. Each amino acid is color coded by confidence level (see section 6.2.2 Ion Table Frame) with the masses for matched a, b and c ions listed in blue and for the matched x, y and z ions listed in red.

Below the “Ion Table Frame” is the “Spectrum View Frame”. This frame is useful for seeing the strength of the ms/ms peaks that PEAKS has set as ions. Here the alignment of the assigned b (blue) and y (red) ions with the entire spectrum corresponding to the selected peptide can be observed. For more information on the “Spectrum View Frame”, see section 6.2.3 Spectrum View Frame.

By clicking on the tabs are the bottom of the page, you can view the corresponding survey scan, the spectrum alignment and the error map. For more information, see section 6.2.4 Spectrum Alignment Frame, 6.2.5 Survey Scan and 6.2.6 Error Map.

3.5 Run Protein Identification

1) Click the PEAKS Search toolbar icon

Or

Select “PEAKS Search” from the “Tools” menu.

2) Enter the settings as shown:

Enter the PTM as shown below by selecting “Set PTM”: 

21
Parameters can be saved and used for future reference by clicking on the “Save Parameter” button. For more information on setting up protein identification parameters see section 7.1 Setting up Protein Identification Parameters.

Click “OK” to commence analysis.

Once the Protein ID file appears in the “Project View Panel”, double click on the file and the results will appear in the “Main Processing Window”:

The “Peptide View” will appear in the “Main processing” window by default. The “Peptide ID List” shows each spectrum for which PEAKS found a matching peptide. Since there may be more than one spectrum that matches a peptide, these spectra would be listed together under a Hit node. Use the ‘+’ and ‘-’ boxes to expand and collapse the node to see the spectra that are listed together. With this dataset, spectra 4 and 5 can both be found under one hit.

The “Peptide Alignment Panel” contains an “Ion Table”,

22
“Spectrum View Frame”, “Spectrum Alignment Frame”, “Survey Scan” and “Error Map”. Click on the “Peptide Details” tab to see a simple alignment between the original de novo sequence, the peptide found in the database and the reconstructed sequence. At the bottom of the “Peptide Details” panel you will see where the selected peptide matches the protein. For more information about these sections see sections 6.2.2 Ion Table Frame - 6.2.6 Error Map.

Click on the “Protein View” tab on the upper left hand side. The following window will appear:

PEAKS 5.1 presents a list of proteins, ranked in descending order from highest score on downward. Clicking on any protein will display the peptides matched to that protein in the bottom pane. In this case the highest scoring protein match is Serotransferrin precursor from bovine. This protein has two matching peptides, which you can see in the “Peptide ID” list when you have selected the “Peptides” tab. To see exactly where the peptide corresponds to the protein sequence, select the “Coverage” tab. The entire sequence of the protein is shown and the matched parts are highlighted in blue. In this case the total matched part accounts for 3.69% of the protein. This information can be found in the “Coverage” column.

3.6 Run PTM Finder
Using the PTM finder, you can identify any additional PTMs and increase the coverage of the proteins that we have found. It is important to note that the PTM Finder can only be applied to a Protein ID results file. As it is very time consuming to run Protein ID with many PTMs, this allows searching for more PTMs in less time. Make sure that you click on a Protein ID result before performing a PTM Finder search.

1) Click the PTM Finder toolbar icon  
   Or  
   Select “PTM Finder” from the “Tools” menu.

2) Enter the settings as shown:
Enter the PTM as shown below by selecting “Set PTM”:

Finder Parameters. Click “OK” to commence analysis.

After the PTM Finder search is complete, double click on the result node. The “Peptide View” window will appear:

Saving the parameters for future reference is achieved by clicking on the “Save Parameter” button. For more information on setting up PTM Finder parameters see section 9.1 Setting up PTM.
The results will be displayed in the same format as was seen for Protein ID. Recall that the Protein ID search identified spectra 1, 3, 4 and 5. The PTM finder search also displayed spectra 2 with the addition of deamidation on N.

### 3.7 Run an inChorus Search

Performing the search with the same data by different search engines is useful both for finding new proteins and confirming others. You can perform and inChorus search using PEAKS Protein ID, X!Tandem, OMSSA, Mascot and Sequest. For this example we will be performing a local search using the X!Tandem and OMSSA search engines. If you have not already set up your search engine preferences, see section 15.1.3 Search Engine Preferences for more instructions.

1) Click on the orbisample.mzxml file

2) Then click the inChorus Search toolbar icon 🌎

Or

Select “inChorus Search” from the “Tools” menu.
The inChorus search window will open. Check the “PEAKS Protein ID” box and select the name. Enter the following settings:

Enter the PTM as shown below by selecting “Set PTM”:

Next, check the “X!Tandem” box.
Enter the following settings:

Finally, check the “OMSSA” box and select the name.
Enter the following settings:

Click the “Ok button. When the inChorus search is complete you should see the following new additions in the “Project View” panel:

- PEAKS 1 [21-Jan-09 11:38]
- X!Tandem 2 [21-Jan-09 11:39]
- OMSSA 3 [21-Jan-09 11:40]
- INCHORUS 4 [21-Jan-09 11:40]

Presented here are individual reports for PEAKS, X!Tandem and OMSSA as well as an inChorus report that compares the individual reports. To see each of these reports, double click on the report that you would like to see in the “Project View” panel.
The “Peptide View” results for the PEAKS Protein ID search can be seen below:

<table>
<thead>
<tr>
<th>ID</th>
<th>Peptide</th>
<th>Sequence</th>
<th>PEAKS(%)</th>
<th>M/Z</th>
<th>Z</th>
<th>M(Calc)</th>
<th>Delta(Mass)</th>
<th>Error(gppm)</th>
<th>File</th>
<th>RT</th>
<th>Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spectrum 1</td>
<td>DYPHYMPSAIK</td>
<td>80.95</td>
<td>803.51</td>
<td>2</td>
<td>1,603,799</td>
<td>-0.000</td>
<td>3,876.00Sample...</td>
<td>0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 2</td>
<td>TSQMDR[1]NQNY</td>
<td>59.39</td>
<td>699.84</td>
<td>2</td>
<td>2,139,662</td>
<td>-0.013</td>
<td>9,967.00Sample...</td>
<td>0.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 3</td>
<td>AHELIMINK</td>
<td>57.37</td>
<td>603.87</td>
<td>2</td>
<td>2,142,930</td>
<td>-0.000</td>
<td>6,088.00Sample...</td>
<td>0.348</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 4</td>
<td>TNDV[1]MIP</td>
<td>55.69</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 5</td>
<td>AMFGR[1]IVF</td>
<td>53.99</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The “Peptide View” results for the X!Tandem search can be seen below:

<table>
<thead>
<tr>
<th>ID</th>
<th>Peptide</th>
<th>Sequence</th>
<th>X!TANDEM</th>
<th>M/Z</th>
<th>Z</th>
<th>M(Calc)</th>
<th>Delta(Mass)</th>
<th>Error(gppm)</th>
<th>File</th>
<th>RT</th>
<th>Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spectrum 1</td>
<td>KYPPPIQMSAK</td>
<td>16.19</td>
<td>603.51</td>
<td>2</td>
<td>1,603,799</td>
<td>-0.006</td>
<td>3,876.00Sample...</td>
<td>0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 2</td>
<td>TSQMDR[1]NQNY</td>
<td>59.39</td>
<td>699.84</td>
<td>2</td>
<td>2,139,662</td>
<td>-0.013</td>
<td>9,967.00Sample...</td>
<td>0.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 3</td>
<td>AHELIMINK</td>
<td>57.37</td>
<td>603.87</td>
<td>2</td>
<td>2,142,930</td>
<td>-0.000</td>
<td>6,088.00Sample...</td>
<td>0.348</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 4</td>
<td>TNDV[1]MIP</td>
<td>55.69</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 5</td>
<td>AMFGR[1]IVF</td>
<td>53.99</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The “Peptide View” results for the OMSSA search can be seen below:

<table>
<thead>
<tr>
<th>ID</th>
<th>Peptide</th>
<th>Sequence</th>
<th>OMSSA(e-value)</th>
<th>M/Z</th>
<th>Z</th>
<th>M(Calc)</th>
<th>Delta(Mass)</th>
<th>Error(gppm)</th>
<th>File</th>
<th>RT</th>
<th>Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spectrum 1</td>
<td>DYPHYMPSAIK</td>
<td>1.41E-12</td>
<td>803.51</td>
<td>2</td>
<td>1,603,799</td>
<td>-0.000</td>
<td>3,876.00Sample...</td>
<td>0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 2</td>
<td>TSQMDR[1]NQNY</td>
<td>59.39</td>
<td>699.84</td>
<td>2</td>
<td>2,139,662</td>
<td>-0.013</td>
<td>9,967.00Sample...</td>
<td>0.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 3</td>
<td>AHELIMINK</td>
<td>57.37</td>
<td>603.87</td>
<td>2</td>
<td>2,142,930</td>
<td>-0.000</td>
<td>6,088.00Sample...</td>
<td>0.348</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 4</td>
<td>TNDV[1]MIP</td>
<td>55.69</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spectrum 5</td>
<td>AMFGR[1]IVF</td>
<td>53.99</td>
<td>600.89</td>
<td>2</td>
<td>1,639,759</td>
<td>-0.037</td>
<td>3,945.00Sample...</td>
<td>1.428</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The inChorus report contains most of the information that is seen in a PEAKS Protein ID results file (7.2 Protein Identification Results). Click on the “Peptide View” tab:

The “Peptide View” of an inChorus report contains the scores received by each search engine involved in the inChorus search. A “-” indicates that the search engine did not find that a protein sequence for that particular spectrum. Notice that while PEAKS Protein ID found spectra 1-5, X!Tandem found 1, 5 and OMSSA found 1, 3, 4 and 5.
Click on the “Protein View” tab:

The “Protein View” of an inChorus report displays the proteins that were found and indicates by checkmarks whether the different search engines found that protein or not. In this case, the X!Tandem and OMSSA searches did not generate any extra results that PEAKS did not find but helped to confirm that the first protein is a good match.

3.8 Perform a SPIDER Search

In this example, spectrum 6 has not been identified with database searching tools. In order to gain more information from our data, we will run a SPIDER search next. For more information about the SPIDER search see section 8. SPIDER Search.

1) Click on the Protein ID Result
2) Next, click the SPIDER Search toolbar icon 🌴
   Or
   Select “SPIDER Search” from the “Tools” menu.
3) Enter the settings as shown:
Enter the PTM as shown below by selecting “Set PTM”:

<table>
<thead>
<tr>
<th>Name</th>
<th>Pm 1 mass</th>
<th>Residue site</th>
</tr>
</thead>
<tbody>
<tr>
<td>K8</td>
<td>104.0300</td>
<td>x</td>
</tr>
<tr>
<td>Lipyl</td>
<td>106.0330</td>
<td>(f)</td>
</tr>
<tr>
<td>Methyl ester</td>
<td>144.0516</td>
<td>(K), [M+H]</td>
</tr>
<tr>
<td>Methylation</td>
<td>144.0516</td>
<td>(K), [M+H]</td>
</tr>
<tr>
<td>O1B label</td>
<td>2.0046</td>
<td>[S]O, [M+H]</td>
</tr>
<tr>
<td>Propionamide</td>
<td>71.0371</td>
<td>[C]</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>162.0470</td>
<td>(K), [M+H]</td>
</tr>
<tr>
<td>Myristoylation</td>
<td>210.1924</td>
<td>[K], [M+H]</td>
</tr>
<tr>
<td>N-acetylglucosamine</td>
<td>786.7598</td>
<td>[C]</td>
</tr>
<tr>
<td>N-acetylglucosaminidase</td>
<td>99.0594</td>
<td>[C]</td>
</tr>
<tr>
<td>N-butyryl-morphol</td>
<td>127.0633</td>
<td>[C], [M+H]</td>
</tr>
<tr>
<td>Oxidation M</td>
<td>157.0567</td>
<td>[M]</td>
</tr>
<tr>
<td>Oxidation N</td>
<td>157.0567</td>
<td>[N]</td>
</tr>
<tr>
<td>Peptide cleavage</td>
<td>228.2357</td>
<td>[NH2]</td>
</tr>
<tr>
<td>Phosphohistidine</td>
<td>380.0658</td>
<td>[P]</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>79.9663</td>
<td>[STK/DC]</td>
</tr>
<tr>
<td>Phosphorylation STV</td>
<td>79.9663</td>
<td>[STV]</td>
</tr>
</tbody>
</table>

Select Fixed PTM:
- Carboxymethyl

Select Variable PTM:
- Deamidation
- Oxidation M

Parameters can be saved for future reference by clicking on the “Save Parameter” button. For more information on setting up SPIDER Search parameters see section 8.1 Setting up SPIDER Parameters. Click “OK” to commence analysis.

After the SPIDER search has completed, double click on the SPIDER results node. The “Peptide View” window will appear. The format is identical to what was seen in the results of a Protein ID search:

Note that all spectra can now be identified by the SPIDER search. Spectrum 6 is well identified.
Clicking on the “Peptide Details” tab will display the protein with its matched peptides in red. SPIDER will also display a reconstructed sequence. See section 8.2 SPIDER Results View for more information.

Click on the “Protein View” tab and then on the “Coverage” tab to see where the SPIDER results overlap with the protein sequence. Note that SPIDER matches are shown in red while matches found by both PEAKS protein ID and SPIDER are shown in magenta:
4. Load Data

4.1 Data Formats
Before loading data files into PEAKS, you must make sure that the data is in an accessible format. PEAKS handles data files in the following formats:

- .ANZ – the zip compressed XML based file format associated with PEAKS 4.5 and earlier
- .BAF, .YEP and folders of .FID files from Bruker instruments
- .D files from Agilent QTOF instruments
- .DAT files created by BSI’s ABI converter software
- .DTA: file format associated with SEQUEST software
- .MGF: file format associated with Mascot software
- .PKL: file format associated with MassLynx software
- .RAW files from Thermo Electron instruments
- .RAW files from Waters QTOF instruments
- .WIFF files from ABI/Sciex QSTAR and QTRAP instruments
- .XML format files using the mzData schema
- .XML format files using the mzXML schema
- PEAKS 5 project directories

4.2 Data Conversion
It is best to import RAW data directly, so that PEAKS can access the complete, unprocessed experimental data including the MS survey scan and retention time information. This will ensure that the PEAKS analysis does not suffer from poor preprocessing.

In order to load RAW data from different vendors, PEAKS may require third-party software to be installed. Please consult the following instructions for third-party software requirements.

4.2.1 PEAKS Project Converter
Projects created using PEAKS 5.0 are different than those produced with 5.1. PEAKS provides a straight-forward project conversion tool that will allow users to quickly enhance existing projects to be supported in PEAKS 5.1. Users comfortable with command line, will find the tool very easy. This section will cater to those unfamiliar with command line, but who wish to convert their necessary projects promptly.
There are two main methods that can be used to generate a PEAKS 5.1 project from an existing 5.0 project.

1. The first method converts a 5.0 project and stores the 5.1 project in the same directory as the original file, with the addition of “51” to the end of the file. Just enter ProjectConverter followed by the PEAKS 5.0 project location and name. For example, if the project is found in your C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project folder, enter

   ProjectConverter “C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project”

   This will produce a new 5.1 project in this same directory, with the name My5.0Project.51.

   The quotation marks (“”) surrounding the location are not necessary in this case, but are necessary if your path contains any spaces, such would be found in use of “C:/My Documents/Projects…”.

2. To produce a new file in a different directory, the only change is the addition of leaving a blank space, and then entering the output directory, such as “C:\PeaksStudio5.1\derbyServer\serverDB\My5.1Project”

   Thus the line will look like

   ProjectConverter “C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project” “C:\PeaksStudio5.1\derbyServer\serverDB\My5.1Project”

   If the new directory does not already exist, the program will create the directory for you. If the directory already exists, entering a dash and the letter ‘-f’ before the original project will force the ProjectConverter to overwrite any existing projects in this location.

   For example:

   ProjectConverter -f “C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project”
   Or

   ProjectConverter -f “C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project” “C:\PeaksStudio5.1\derbyServer\serverDB\My5.1Project”

   You are now ready to call up these projects into PEAKS 5.1.

4.2.2 Agilent Data

PEAKS 5.1 can load native data from Agilent QTOF, provided that the Mass Hunter software is installed on the same computer.

4.2.3 Applied Biosystems (WIFF) Data

WIFF data from Applied Biosystems/Sciex QSTAR (or QTRAP) mass spectrometers can be loaded, provided that the Analyst QS (Analyst 1.4.1 for QTRAP) software and the MSX plug-in are installed on the same computer as PEAKS 5.1. The MSX tool is produced and sold by Infochromics Ltd., and is available (at cost) from Bioinformatics Solutions Inc. Please contact a BSI sales representative to obtain an evaluation or full license.
4.2.4 Applied Biosystems (4700/4800) Data
BSI has created a converter to extract the data from an ABI-Oracle database. If you require this separate, free tool, contact your sales representative. Once installed, you can start up the ABI 4700 Data Extractor from the Start menu.

4.2.5 Bruker Data
PEAKS 5.1 can load data from Bruker mass spectrometers provided that the CompassXport software is installed on the same computer. If loading .fid files, which are stored in a network of folders, select the top level folder to load them all at once.

4.2.6 Shimadzu Data
Shimadzu mass spectrometer data can be loaded, provided that the Shimadzu software is installed on the same computer as PEAKS 5.1.

System Requirements
This extractor can be installed on the same machine as ABI 4700 Explorer and the Oracle database (we will call this machine the 4700 SERVER in the following instructions) or another machine that has direct network access (we require that there is no firewall or proxy) to the 4700 SERVER. Windows 2000 or Windows XP is recommended for use with this tool.

Configuration
Before using the ABI 4700 Data Extractor, it must be configured. To do so, choose “Settings” from the “File” menu. Configuration requires the following:

4700 SERVER Name or IP Address: input “localhost” if the Extractor is running on the 4700 SERVER (this is the default value), otherwise enter the IP address of the 4700 SERVER.

The socket used by the 4700 SERVER: this is the port that the Oracle database listens to (the default is 1521).

Username to access the Oracle database: most likely we do not need to change this (the default is “tsquared”).

Password to access the Oracle database: mostly likely you do not need to change this one either.

Data extraction procedure
1. Load Spot Set List from the database: (Do it via menu File | Load Spot Set List). The extractor will export the peak list of a spot set into a PKL file.
2. Open a Spot Set: (menu File | Open Spot Set) Spot Set Chooser will help the user to choose a spot set. After selecting a spot set, click 'OK' to open it. The job run information of a spot set will be shown.

3. Select a job run: There is a button to select before each job run. Only the MS/MS job run can be selected for export, as the precursor information is needed. Select a job run and click 'Convert' to do the extraction.

4. Choose a filename to save: After clicking the 'Convert' button, the user needs to input a file name and the peak lists of the selected job run will be exported.

4.2.7 Thermo Data
RAW data from Thermo Electron mass spectrometers can be loaded, provided that the XCalibur software is installed on the same computer as PEAKS 5.1.

4.2.8 Varian Data
A conversion tool is embedded into Varian’s data acquisition software which allows the conversion of Varian raw data into .pkl files which can be immediately read by PEAKS.

The .trans type data (raw) is converted in Varian programs by clicking "File", "Save As" and selecting the .pkl file format or by clicking "File", right clicking "Export" and selecting ".pkl". If you are viewing a chromatogram with the Varian software, all the spectra data in the viewed chromatogram is converted to the .pkl format. Likewise, if you are viewing a single spectrum and choose to convert the data, only the viewed spectra will be converted.

4.2.9 Waters/Micromass (MassLynx) Data
PEAKS 5.1 can import RAW data from Waters/MicroMass QTOF instruments using a utility called wolf.exe (originally created as part of the Sashimi Project) to access MassLynx libraries and convert the data. PEAKS provides a version of wolf.exe compatible with MassLynx 4.1. If you need a different version of wolf.exe, please visit:

www.bioinfor.com/products/peaks/support/watersmicromass.php

Additionally, you must make sure that the following MassLynx libraries are installed on the same computer as PEAKS and wolf.exe:

- DACServer.dll
- Genutil.dll
- MetaGD32.dll
- raw.dll
- securityAccess.dll
- securitySettings.dll
- securitySignature.dll
4.3 Create a New Project

1) To create a new project, select “New Project” from the file menu or using the “New project” icon on the toolbar. The following window will open:

2) Create a name for your project and click “Browse” to select the where you would like to save the project.

3) To add data files to sample 1, click the “Add data files” button. Browse for the data file and click the “Open” button. Once the data file appears, select the “Instrument Vendor” and “Instrument type” that was used to generate the experimental data from the drop-down lists. Select “All Instruments” from the “Instrument Vendor” drop-down list to view general instrument types in the “Instrument Type” drop-down list. If you would like to apply the same instrument configuration to all of the files in the sample, or to all of the samples in the project click on the “whole sample” button or whole project button, respectively.

4) To add another sample, click on the “Add Sample” button. To add a data file to sample 2 click on the “Add data files” button. Select the instrument vendor and type from the drop-down menus unless you had previously applied the instrument configuration to the whole project in step 3.
5) To delete a sample or data file, select the appropriate node (sample or data file) and click the “Delete” button. You can also change the order of the samples within a project or data files within a sample using the “Up” and “Down” buttons.

6) Click the “OK” button once all data files and samples are added to the project.

7) The project will appear in the “Project View” window. The “字样” symbol indicates that the file is still loading (see example below).

The “字样” symbol indicates that the file has finished loading (see example below).

4.4 Add Data to an Existing Project

1) Select “Add data” from the file menu or “字样” from the task bar. The original project window will open. You can add more files to an existing sample or create additional samples. You will need to select the instrument vendor type. For more information on adding files/samples or setting up the instrument configuration, refer to section 4.3 Create a New Project.
4.5 Open / Close a Project

To open a stored project, select “Open Project” or “Open Recent Project” from the file menu or “ ” from the task bar. To close a project that you are working on, select “Close Project” from the file menu or “ ” from the task bar.

4.6 Delete and Hide Results

Results and filtered results can be hidden or deleted by clicking right clicking on a results file:

Hidden results can be brought back by right clicking on the data file and selecting “Show Reports”. Check the results that you would like to view.

You can delete results, filtered result, fractions, and samples. Note that deleting the latter two will delete any results that depend on them as well. This can be the case even if they only depend on those results partially. While hiding results can be reversed, deleting is permanent.

4.7 Change the Location of Saved Projects

Projects are saved in the location that is listed in your “Preferences” window. To modify your preferences, select the “Preferences” toolbar icon or select “Preferences” from the “Window” menu. Select “General” on the left hand side of the window. The default “Output Directory” and “Project Folder” locations are listed in the “Default Output Directory” panel. Please note that the defaults seen here may differ from your default locations depending where you downloaded PEAKS. Click on the “Browse” buttons to change either of these locations.
You can also change the location of your projects on a project by project basis by selecting a new “Project Location” when setting up a new project as seen below:

4.8 Orienting Yourself

4.8.1 Project View Panel

This frame appears in the upper left hand corner, displays the organization of a particular project (if applicable) or simply of a data file. Use the ‘+’ and ‘-‘ boxes to expand and collapse the project in order to access the data file that you want to analyze. Make sure the data file to be analyzed is selected. If you select a project, all of the samples will be analyzed. If you select a sample, all of the files within that sample will be analyzed.

Note: Any features that cannot be performed when a specific node is selected will be grayed out. In the example below, you cannot run the PTM finder when the raw file is highlighted; you must have a PEAKS Protein ID selected in order to run the PTM finder.

4.8.2 Properties Panel

PEAKS reads and tracks information about the experiment for use in the analysis and for future reference. Once the data file has loaded, click on the properties tab in the bottom left hand corner. If any information cannot be found in the file, PEAKS will prompt you to enter this information.
4.8.3 Raw Spectrum View
Opening the raw file in PEAKS will display the following graphs in the “Main Processing Window”.

The “MS” tab is selected by default and represents the precursor scan. On the left hand side of the screen is the total ion current (TIC). Depending on how the file was generated there may be simply a list of spectra and not a TIC graph. The retention time is plotted against the vertical axis. Clicking on the TIC graph will move the red line and display the ms spectra to the right of the TIC graph that corresponds to the selected retention time. Alternatively, use the up and down arrows, found on the keyboard, to move through the TIC. If the MS2 scans is available, it will be displayed below the corresponding ms scan.

To zoom either on the X or Y axes, select the “2X” or “2Y” buttons, respectively. To scroll in even more, click the button on the left of your mouse and drag the arrow to the side. To increase the intensity of the peaks, use slide the scroll bar on the left hand side, up and down. Selecting the “1:1” button will bring you back to the original image where the entire spectrum is visible.

Scrolling over the spectrum will display the m/z ratio and the height/intensity (as a percentage of 100) of the particular peak under the spectrum view on the right hand side (see the box highlighted in red below).

You can use the profile and peak buttons to switch the spectrum view from profile mode to peak mode and vice versa.
The MS/MS tab gives detailed information about each tandem spectrum:

Each of the spectra in the data file will be listed in the left most panel under the name of the data file. Clicking on one of the spectra will display the results that have been generated for that spectrum in the top right hand panel (as seen in the example above). Before any results files have been generated, the top panel will look like this:

More information can be found about the spectra under the “Info” tab. You will find information about the retention time, where to find the spectra on the TIC graph, the number of peaks and the fragmentation type (if available). You will also find an overview of the results that were found for that spectrum in the results files.

The largest panel displays the MS/MS and below you will find the corresponding MS spectra under the “Survey” tab. Information about navigating through the MS and MS/MS spectra can be found above in the section describing the “MS” tab.
5. Data Refinement

Since mass spectrometry data most often contains noise and redundant data, it makes sense to filter the data before analysis. This will increase the quality of the results, while saving time spent on database searching and de novo sequencing.

When PEAKS Studio or Client are connected to a PEAKS Online server, you will also save time by uploading smaller, preprocessed data. Data refinement can be done locally, before uploading to the server.

5.1 Run Data Refine

To begin the refinement of data from a whole MS/MS run:

1) In the “Project View Frame”, select the data file(s) containing the data that you wish to refine.

2) Click the Data Refine toolbar icon 

   Or

   Select “Data Refine” from the “Tools” menu.

   The Data refinement options window will appear:

3) Choose the data refinement tools you wish to use by clicking the “yes” radio button next to each one. See the information below to help you decide on proper refinement parameters.
5.2 Data Refinement Parameters

5.2.1 Merging Scans
In DDA mode, a mass spectrometer will often produce several tandem ms (MS/MS) scans of the same peptide. To increase the intensity of real signal peaks within these scans and to reduce the size of the whole data set, it makes sense to merge MS/MS scans of the same peptide together. To avoid improper merging (of MS/MS scans of different peptides) we make sure that the measured parent ion masses of these peptides are very close and that they have similar retention times in the LC column. The units here are m/z values in Daltons or ppm. For retention time, we use whatever units are recorded in the data file (usually minutes or seconds).

5.2.2 Precursor Charge Correction
Since a mass spectrometer measures mass-to-charge ratios, we must know the charge on a peptide before we can determine its mass. The standard method of finding the charge is to look at the spacing of the isotope ladder in the survey scan. However many Ion-Trap instruments do not have enough resolution for this. So PEAKS will look at the MS/MS data to determine if it's charge 1+, 2+ or 3+. For data where the survey scan is available, PEAKS will examine the precursor ion’s isotope distribution to confirm or correct the charge assignment. Type in the boxes to set a range of charges. Only spectra that fit in this range will be considered for analysis.

5.2.3 Filtering MS/MS Scans
Scans of contaminants and electrical noise should not be included in analysis. Removing them from the data set will save time, and reduce the risk of random matches to the database. PEAKS offers an effective tool for removing these low quality MS/MS scans. Type in the boxes to set ranges of retention time and m/z ratio. Only peaks between these values will be considered for analysis. Additionally, PEAKS examines the MS/MS spectrum to determine its quality. The quality filter is based on four characteristics: signal to noise ratio over MS/MS, number of peaks after pre-processing, sum of all peak intensities and length of the longest simple sequence tag that can be generated. You can choose a threshold of quality score (a value from 0 to 1) for accepting a scan. We recommend a quality filter of 0.65.

5.2.4 Preprocessing MS/MS Scans
This section deals with deconvolution (de-isotoping), centroiding and noise filtering within the MS/MS data. Preprocessing can save hard disk space or upload time. But make sure to have the original data available in case you need to refer to it later.

To see how your data is changed after data refinement, refer to the data properties window.

5.3 Data Preprocessing Results
To view the result of data pre-processing, click on the MS/MS tab on the spectrum view. In following example, the spectrum (m/z = 473.70502) results from raw spectra (m/z = 473.71 and m/z = 473.7).
6. de novo Sequencing

6.1 Setting up Auto de novo Sequencing Parameters

1) In the “Project View Frame”, select the data file(s) or project containing the spectra that you wish to sequence by auto de novo.

2) Click the automatic de novo toolbar icon 🗂️
   Or
   Select “auto de novo” from the “Tools” menu.

The auto de novo parameters dialogue window will appear:

3) To change any of the following parameters, now is the time:
6.1.1 Mass Options

*Parent mass error tolerance:* Determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. As you have previously selected your instrument, PEAKS will provide the suggested error tolerances. Type a tolerance in the textbox and choose units from the drop-down list. Using PPM allows for larger errors at larger m/z values. PEAKS will be very stringent concerning this value, so new PEAKS users should try setting this a little higher than past experience may suggest, if sensitivity is a concern.

*Fragment mass error tolerance:* Determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. As an instrument has previously been selected, PEAKS will provide suggested error tolerances. Type a tolerance in the textbox. Again, new PEAKS users should try setting this a little higher than past experience may suggest.

6.1.2 Enzyme Options

*Enzyme:* Tell PEAKS what type of enzyme was used to digest the sample. Choose from a drop-down list of enzymes, or if your enzyme is not in the list, click the "New Enzyme" button. You can then input the name of the new enzyme.

*Digest Rules:* Enter the amino acid that is found at the end of the peptide. Put set brackets {} around a residue to denote any amino acids except for those that are within the brackets. Select the “Advanced” button if your digest rules are more complicated. Select the radio box “Select peptides that satisfy at both ends” if you require that your peptide was cut by the enzyme you chose at both ends.

6.1.3 PTM Options

*Selecting fixed and variable PTMs:* The “PTM Options” list tells PEAKS what types of post-translational modifications to include in its analysis. To view additional modifications, select the “Show unimod” box. If a desired PTM does not appear on the list or is different than what is listed, select the “New PTM” button and the “PTM Editing” window will open. Fill in the information pertaining to your PTM. To select a PTM as Fixed or Variable, drag the PTM into the Fixed Modification or Variable Modification box. If you drag over an incorrect PTM, simply drag it back to the “PTM Options” list.

*Max variable PTM per peptide:* To reduce uncertainty, limit PEAKS' de novo sequencing ‘vocabulary’ by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

6.1.4 General Options

*Report up to (# peptides):* Set how many peptide sequences PEAKS will report from its de novo sequencing analysis.

*Preprocess your data “on the fly” before auto de novo:* PEAKS has its own built-in preprocessor for removing noise, centroiding and deconvolution. Check this box to turn preprocessing on.
BSI highly recommends using PEAKS to preprocess all data, as opposed to using instrument vendor software, if the data is to be used by PEAKS. PEAKS preprocessor should not be used on data that has already been pre-processed as this will have adverse effects on the results (unless it is ion-trap data).

Note: If you have already pre-processed your data in the data refinement step, you do not need to do this again.

4. After setting parameters, you can save them for future use. Click the "Save Parameters" button at the top of the window, and choose a name for future reference when prompted. Any parameters that are saved will be available in the drop-down list at the top of the window. To see what's inside, select one and the parameters boxes will be populated.

5. Press the "OK" button to initiate de novo sequencing.

6.2 de novo Sequencing Results

Once de novo sequencing is finished, the following window will open:
6.2.1 Peptide Candidates Frame

PEAKS displays the peptide sequence candidates at the top of the screen in the “Peptide Candidates Frame”. You can sort the results by clicking on any of the titles of the columns. For example, to sort the peptide sequence candidates by ID click on “ID”. Note that all of the peptides that have the same ID have the same mass, charge, retention time and quality score (See section 5.2.3 Filtering MS/MS Scans for more information on how the quality score is generated).

The following table describes the contents of the columns in the “Peptide Candidates Frame”:

<table>
<thead>
<tr>
<th>ID</th>
<th>A unique identifier for the MS/MS spectrum. This differs from a scan number since we may have merged several scans together.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>The sequence of the peptide (including modifications if present) as determined by de novo sequencing.</td>
</tr>
<tr>
<td>TLC</td>
<td>Total local confidence (the confidence that we have in the peptide sequence). It is calculated by adding the positional confidence for each amino acid in the peptide sequence.</td>
</tr>
<tr>
<td>ALC</td>
<td>Average local confidence (the confidence that we have in the peptide sequence). It is calculated by adding the positional confidence for each amino acid in the peptide sequence and dividing by the total number of amino acids.</td>
</tr>
<tr>
<td>Rank</td>
<td>The sequences for a particular spectrum (ID) as sorted by score (TLC).</td>
</tr>
<tr>
<td>m/z</td>
<td>The measured mass/charge value, in Daltons, for the peptide.</td>
</tr>
<tr>
<td>Z</td>
<td>The calculated charge value for the peptide.</td>
</tr>
<tr>
<td>Mass</td>
<td>Calculated using the measured m/z and calculated z, we use this as the experimental mass of the peptide.</td>
</tr>
<tr>
<td>File</td>
<td>The name of the file.</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time (elution time) for the peptide as recorded in the scan header.</td>
</tr>
<tr>
<td>Scan</td>
<td>The scan number.</td>
</tr>
<tr>
<td>Quality</td>
<td>A value from 0 to 1 estimated from the spectrum to refer to spectrum quality. Attributes like signal to noise, total intensity, and spectrum tagging are used.</td>
</tr>
<tr>
<td>Scan Mode</td>
<td>Mode that the scan step was performed in.</td>
</tr>
<tr>
<td>Frag. Mode</td>
<td>Mode that the fragmentation step was performed in.</td>
</tr>
</tbody>
</table>
The columns themselves are customizable. To change the columns are displayed click on the “_cols” button.

Confidence Scores
Next to the proposed sequence candidates, the auto de novo “Total Local Confidence” (TLC) and “Average Local Confidence” (ALC) confidence scores are shown. The confidence scores for each amino acid (that is, confidence that the correct residue in each position has been identified) are represented by color coding. Red represents a very high confidence (greater than 90%), purple represents a high confidence (80 to 90%) blue represents a medium confidence (60 to 80%) and black represents a low confidence (less than 60%). For more detailed positional confidence, place the cursor over the sequence of interest and right click “Show Positional Confidence”. A “Position Confidence Table” will appear, showing the confidence that each amino acid/pair of amino acids are correct.

Sequence Tags
Right click on a peptide in the “Peptide Candidates Frame” and select “Show Sequence Tag”. If the score threshold is set at 0.0, all of the amino acids in the peptide sequence will be displayed. Increasing the “Score Threshold” will display a mass in square brackets if the amino acids do not satisfy the score threshold.

Modifications
Consider the following sequence: DW[1]C[1]SFTDAENVQALAR
The number 1 in square brackets refers to where a modification may occur. If you forget what modifications you selected before running de novo, click to the “Properties” tab.

The fixed modification is set to [1]58.005478@[CKW]. In the sequence above, the modification has been made to the W as well as the C. The colors assigned to the [1] follow the same confidence scores as the amino acids themselves.

Refer to the above section on “Confidence Scores” for more information on color coding.
6.2.2 Ion Table Frame

The “Ion Table” shows the proposed ions with their corresponding masses. To add additional ions to the ion table, see section 15.1.4 Ion Editor Preferences.

If an ion is found in the corresponding spectrum, it must first pass two criteria before being displayed in a specific color (blue for N-terminal ions and red for C-terminal ions). It must be found within the mass error tolerance chosen by the user and must have an intensity of greater than 2% of the ion with the greatest intensity.

6.2.3 Spectrum View Frame

The “Spectrum View Frame” is found below the “Ion Table” and displays a graphical representation of the spectrum. The peptide that corresponds to the spectrum in the “Spectrum View Frame” is displayed in the “Input Sequence” box. Use the drop-down to select other peptides that have the same ID.

Scrolling over the spectrum will display a “tooltip” in the new window that will display the m/z ratio and the height/ intensity (as a percentage of 100) of that particular peak. Both the m/z ratio and the height of the peak can also be found under the spectrum view on the right hand side.

To zoom either on the X or Y axes, select the “Zoom X” or “Zoom Y” buttons, respectively and then use the wheel on your mouse to move around the graph. Selecting the “1:1” button will restore settings to view the entire spectrum on the screen.

You can use the profile and peak buttons to switch the spectrum view from profile mode to peak mode and vice versa. The scrollbar on the left acts to increase and decrease the intensity of the peaks, where the scrollbar on the right acts to zoom in to display the monoisotopic peaks.
6.2.4 Spectrum Alignment Frame

Clicking on the “Alignment” tab will display the “Spectrum Alignment Frame”. This frame always shows the whole spectrum and is used as a tool to help us navigate the spectrum view frame. A blue bar along the horizontal axis of the alignment view indicates the range of the spectrum view in the Spectrum View Frame. This frame will show you how the proposed ions align with the spectrum. By default, the Spectrum Alignment Frame displays b-ions and y-ions. The b ions are shown right to left in blue, while the y ions are shown left to right in red.

6.2.5 Survey Scan

Clicking on the “Survey” tab will display the corresponding precursor ion spectrum. The buttons that appear in this section are the same as those that are explained above in the “Spectrum View Frame” section.

6.2.6 Error Map

Click on the “Error Map” tab. The m/z ratio is displayed on the y axis and the error is listed on the x axis in Daltons. The “Error Plot” displays the confidence that is assigned to each ion. The most confident results lie on the centerline. Clicking a cell or column in the Ion Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.

6.3 Manual de novo Sequencing

PEAKS 5.1 provides a set of tools to help you sequence a peptide, using graphic cues from the spectrum.

6.3.1 Manual de novo Graphical User Interface

The figure below shows the main panels related to manual de novo. The five main panels are indicated in the figure below:
The panels are briefly described below:

The “Result Panel” shows all the sequencing results. The results of manual de novo are listed in the sub-tree with root “Manual de novo”.

The “Spectrum View Panel” shows a graphical representation of the spectrum: the peaks in the spectrum, the user-selected peaks and assigned ions. We can pick a peak on the panel and assign ions or tags to it in Manual de novo.

The “Alignment View Panel” shows how the proposed ions assigned in manual de novo align with the spectrum. By default, the “Spectrum Alignment Frame” displays b-ion and y-ion. The b ions are shown right to left in blue, while the y ions are shown left to right in red.

The “Ion Table Panel” shows the proposed ions with their corresponding masses. The default Ion Table will display immonium, b, b-H2O, a, c, y, y-H2O, z, z’ and y(2+) ions.
The “Tag Panel” will appear when you search tags or ions in the spectrum. You can select the tags in the list using the “Select” button. Clicking the “Apply” button will add the selected tags to the sequence candidate.

To create a new peptide candidate for sequencing:
1) Right click in the “Results Panel”. This will bring up a pop-up menu.

2) Select “New candidate for manual de novo” from the pop-up menu. A new candidate will be created under the ‘Manual de novo’ heading. The new candidate will not have been sequenced, so it will be represented by the mass of the peptide less the mass of water (see an example below).

![Image](image_url)

Note: The popup menu will not be accessible if you have highlighted any of the results in the “Results Panel”.

### 6.3.2 Manual de novo Operations

Note: All operations occur in the Spectrum View Frame of the Main Processing Window.

When the mouse is placed in the Spectrum View Frame, a green (by default) arrow follows the movement of the mouse. This is the Position Bar and it is used as a cursor for all manual de novo operations. The cursor’s position on the m/z scale is enumerated on the top of the Position Bar.

#### Selecting a peak
To select a peak, click on it. A blue (by default) arrow, called the Freeze Bar, indicates the selected peak.

Alternatively an ion peak can be selected by clicking on its corresponding cell in the Ion Table.
Measure the m/z difference between two PEAKS
Select a peak (blue arrow) with the Freeze Bar and move the mouse to the left or right. Hold the Position Bar (green arrow) above another peak. The number in the Position Bar is the difference between the two PEAKS (in the example below the difference is 80.99109).

Deselect a peak
Double click anywhere in the Spectrum View Frame

Zoom in on part of the spectrum
In the Spectrum View Frame, click and drag the mouse horizontally. The selected area will be shown in the Spectrum View Frame. Click on the magnifying tool "🔍" to display a panel with the spectrum zoomed in. The peak which is selected by the Position bar will be highlighted in the zoomed panel in green.

Click on the magnifying tool again to remove the zoomed panel.

Setting/removing ions to/from a peak
Select a peak, then right click the mouse anywhere in the Spectrum View Frame. Select “Set y-ion” from the pop-up menu to designate the peak as a y ion, or “Set b-ion” from the pop-up menu to designate the peak as a b ion. Click on “Remove ion” to remove the ion that you have previously set.

Select “Set other ions” from the pop-up menu to view the “Ion Editor” dialog box. The Ion Editor dialogue allows you to add or remove ion designations to/from...
a peak. Select either “C Term Ion” or “N Term Ion” to see the C and N terminal ions respectively. Then select an ion from the ion choice list and press the “Add” button to add it to the selected ion list. Remove an ion from the selected ion list by selecting it and pressing the “Remove” button. Click “Apply” to apply the changes to the selected peak.

After setting an ion, both the alignment view and the peptide sequence candidate name (as displayed in the peptide candidate frame) will change to reflect the mass remaining to be sequenced on either side of the ion.

In the example below, the selected peak at 416.2848 m/z was designated as a y-ion.

Note that the manual de novo candidate information is updated in the results panel, ion table and alignment view panel. The selected ions are also highlighted in the spectrum view panel.
After setting two ions, PEAKS 5.1 will estimate the residue found between them (if a residue corresponds closely to the mass difference). The peptide sequence candidate name (as displayed in the peptide candidate frame) will change to show the residue and the mass remaining to be sequenced on either side of the residue.

**Searching the left or right side of the spectrum for the first/last y or b ion**

Select a peak, then right click the mouse anywhere in the Spectrum View Frame to trigger the popup menu. From the menu, select either “Left tags” or “Right tags”. PEAKS will select the appropriate terminal tags and show them in the “Tag Panel” frame (see below).

You can test the suitability of a tag by highlighting it in the “Searched Tags” panel; the corresponding information for the tag will be shown in the Spectrum View panel, the Ion table and the Alignment frame panel. You can insert one or more tags by highlighting the desired tags, clicking “Select” to move them into the “Selected Tags” panel and then clicking “Apply”. Press the “Cancel” button at any time to exit the search and discard any changes.
Search a sequence tag
Select a peak with a defined ion (i.e. an ion that has been labeled with a peptide). Right click to trigger the popup menu, then select “Right tags” or “Left tags” to search peptide tags either to the right or left of the selected peak. PEAKS will select the appropriate tags and show them in the “Tag Panel” frame. You can test the suitability of a tag by highlighting it in the “Searched Tags” panel; the corresponding information for the tag will be shown in the Spectrum View panel, the Ion table and the Alignment frame panel. You can insert one or more tags by highlighting the desired tags, clicking “Select” to move them into the “Selected Tags” panel and then clicking “Apply”. Press the “Cancel” button at any time to exit the search and discard any changes.

Undoing an edit
If you have made an error in your sequencing it is possible to undo the change. With the Peptide candidate still selected in the “Results panel”, right click the mouse and select “Undo” to return to the previous peptide sequence. You can click this button multiple times to return to earlier stages in your edit.

Redoing an edit
With the Peptide candidate still selected in the “Results panel”, right click the mouse and select “Redo” if you have undone one too many changes. You can click this button multiple times to proceed to later stages in your edit.
7. Database Search

7.1 Setting up Protein Identification Parameters

1) In the “Project View Frame”, select the data file(s) or project containing the spectra that you wish to identify using database search.

2) Click the Protein Identification toolbar icon or
   Select “PEAKS Protein ID” from the “Tools” menu.

The Protein Identification Parameters dialogue window will appear:

3) To change any of the protein identification search parameters, now is the time.
7.1.1 Mass Options

*Parent mass error tolerance:* Determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. Type a tolerance in the textbox and choose units from the drop-down list. Using PPM allows for larger errors at larger m/z values. PEAKS will be very stringent concerning this value, so new PEAKS users should try setting this a little higher than past experience may suggest, if sensitivity is a concern.

*Fragment mass error tolerance:* Determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. Type a tolerance in the textbox. Again, new PEAKS users should try setting this a little higher than past experience may suggest.

*Precursor mass search type:* If the precursor mass is monoisotopic value, check monoisotopic. Check average, otherwise.

7.1.2 Enzyme Options

*Enzyme:* Indicate which type of enzyme was used to digest the sample. Choose from a drop-down list of enzymes. Note that you cannot delete or change the details of a built-in enzyme and therefore the “Delete enzyme” button and the “Digest Rules” panel will be grayed out. If your enzyme (or combination of enzymes) is not in the list, click the “New Enzymes” button. You will then be able to enter a name for your enzyme, digest rules (see below) and select if you would like to find proteins that satisfy the rules at both ends. This option is grayed out for built-in enzymes.

*Digest Rules:* This is how you specify where your enzyme will cleave the protein between two amino acids to create peptides. The letter X denotes ‘any amino acid in this position’, while {set brackets} indicate any amino acid except the one in the brackets. Clicking on the “Advanced” button will open a new window which will allow you to be more specific with your digest rules.

7.1.3 General Options

*Max missed cleavages:* determine the most missed cleavages to allow, internal to the peptide, in a *de novo* sequence. For instance, setting this to 2, and Trypsin as the enzyme, then PEAKS will return *de novo* sequences with up to 2 R's or K's internally.
Preprocess before auto de novo: PEAKS has its own built-in preprocessor for removing noise, centroiding and peak charge recognition from MS/MS data. Check this box to turn preprocessing on.

7.1.4 PTM Options

PTM options: This list tells PEAKS what kind of post-translational modifications to include in its analysis. Drag the desired PTM into either the “Fixed Modification” or “Variable Modification” box. If the desired PTM is not in the list, first check the “Show Unimod” box to show additional PTMs. To create a new PTM click on the “New PTM” button. The following window will appear:

Fill in the following information:

Name: this name will appear in the PTM list for future use after it is saved.

Monoisotopic mass: the mass that the residue gains or loses as a result of the PTM. Enter this value numerically.

Neutral loss mass: the mass that the modified residue loses as a result of fragmentation. Ex. 28 would signify a loss of 28 Daltons. This is optional.

Chemical formula: the chemical formula of the PTM. This should correspond to the mass listed above. This is optional.

Residues that can be modified: Enter residues that can be modified anywhere, residues that can only be modified if they are at the N-or C-terminus or in the middle only.

Rule: Enter comments for reference. This is optional.

Please note that you can also configure your PTMs in the “Configuration” panel. See section 15.2.2 PTM Configuration for more information.

Max variable PTM per peptide: To reduce uncertainty, limit PEAKS' de novo sequencing 'vocabulary', by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).
7.1.5 Database Options

**Database to search:** Select from this drop-down list, one of the FASTA databases configured in PEAKS. To edit an already existing database, click on the “Edit Database” button. If the desired database is not in this list, click the "New Database" button. Note that you can also set up a new database in the “Database Configuration” window. The configuration window is the only place that you can delete databases that you have created. For more information on setting up new databases see section 15.2.3 Database Configuration.

**Taxonomy selection:** This list displays the taxa you have chosen for your search. If the database selected has taxon information available, you can click on the “Set taxa” button. Otherwise, the whole database will be searched. The selections correspond to established hierarchy -- i.e. selecting 'Mammalia' will search all of 'horse, cow, rat, mouse, human, etc.

**Paste FASTA sequences:** If you already know the sequence of the protein(s) you are looking for, select “Paste fasta sequences” and paste the sequence in the space provided in fasta format. Alternatively, if you want search the same sequence regularly, it is recommended to simply create a small text file and configure it as a database for PEAKS.

7.1.6 Advanced Options

PEAKS needs to have some de novo sequences before database searching since PEAKS uses sequence tags to perform database searching. As such the option of doing de novo prior to protein ID is presented here. In most cases, the same values for instrument, error, enzyme and PTM can be used in de novo and in protein ID, but you have the option of using one of your saved de novo parameter sets for the de novo portion. Select one from the drop-down list.

4) After setting up parameters, we can save them for future use. Click the "Save Parameters" button, and choose a name for future reference when prompted. Any parameters that you save will be available in the drop-down list at the top of the window. To see what’s inside, just select one, and the parameters boxes will be populated.

5) Press the “OK” button. If you have already performed de novo sequencing, the database search will commence automatically. If you have not previously performed de novo sequencing, the auto de novo process will appear first in the task queue. Once de novo sequencing is finished the database search will begin.
7.2 Protein Identification Results

7.2.1 Peptide View

Once PEAKS is finished searching the database, the “Peptide View” window will open by default:

The “Peptide View” window summarizes the results for each MS/MS spectrum. All peptides that match to each spectrum are displayed. By default the spectra are listed by ID in the “ID” column with the corresponding peptide sequence in the “Sequence column” beside. In certain cases, one peptide can correspond to more than one spectrum. These spectra are then listed in the “ID” column under a heading entitled “Hit”. Click on “+” to expand the view to see all of the spectra that can be matched by the same peptide.

The table below describes the contents of the columns in the “Peptide View Window”:

<table>
<thead>
<tr>
<th>ID</th>
<th>A unique identifier for the MS/MS spectrum. This differs from a scan number since we may have merged several scans together.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>The amino acid sequence of the peptide. PTMs are listed in [square brackets].</td>
</tr>
<tr>
<td>Score</td>
<td>PEAKS’ probability score.</td>
</tr>
<tr>
<td>m/z</td>
<td>The measured mass/charge value, in Daltons, for the peptide.</td>
</tr>
<tr>
<td>z</td>
<td>The calculated charge value for the peptide.</td>
</tr>
<tr>
<td>Mr (Calc)</td>
<td>The sum of the theoretical mass of the residues that form the identified peptide sequence from the database.</td>
</tr>
<tr>
<td>Delta (Mass)</td>
<td>The difference between Mr(Calc) and Mass, in Daltons.</td>
</tr>
<tr>
<td>Error (ppm)</td>
<td>The difference between Mr(Calc) and Mass, ppm.</td>
</tr>
<tr>
<td>File</td>
<td>The name of the file.</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time (elution time) for the peptide as recorded in the scan header.</td>
</tr>
<tr>
<td>Scan</td>
<td>The scan number.</td>
</tr>
<tr>
<td>Quality</td>
<td>A value from 0 to 1 estimated from the spectrum to refer to spectrum quality. Attributes like signal to noise, total intensity, and spectrum tagging are used.</td>
</tr>
<tr>
<td>Scan Mode</td>
<td>Mode that the scan step was performed in.</td>
</tr>
<tr>
<td>Frag. Mode</td>
<td>Mode that the fragmentation step was performed in.</td>
</tr>
</tbody>
</table>

The columns themselves can be customized. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any one of them to show or hide a column. These settings will apply to all your reports.

Peptide Alignment

Click on the “Peptide Align” window. This will look very similar to the de novo results window. You will see the “Ion Table” which shows the proposed ions with their corresponding masses. To the right of the “Ion Table” is the “Error Plot” which displays the confidence that is assigned to each ion. The most confident results lie on the centerline. Clicking a cell or column in the Ion
Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.

Underneath the “Ion Table is the “Spectrum View Frame” which displays a graphical representation of the spectrum. The peptide that corresponds to the spectrum in the “Spectrum View Frame” is displayed in the “Input Sequence” box. Note that this is a drop-down menu so that you can select other peptides that have the same ID (if applicable). Scrolling over the spectrum will display a “tooltip” that will display the \( m/z \) ratio and the height/intensity (as a percentage of 100) of that particular peak. Both the \( m/z \) ratio and the height of the peak can also be found under the spectrum view on the right hand side.

To zoom either on the X or Y axes, select the “Zoom X” or “Zoom Y” buttons, respectively and then use the wheel on your mouse to move around the graph. Select the “Slide X” button and then use the wheel on your mouse to move around the graph. You must ensure that you are sufficiently zoomed in on the X axis to use the “Slide X” button. Selecting the “1:1” button will bring you back to the original image where you can see the entire spectrum on the screen.

You can use the profile and peak buttons to switch the spectrum view from profile mode to peak mode and vice versa. The scrollbar on the left acts to increase and decrease the intensity of the peaks, where the scrollbar on the right acts to zoom in to display the monoisotopic peaks.

Finally at the bottom of the screen is the “Spectrum Alignment Frame” which is used as a tool to navigate the “Spectrum View” frame. A blue bar along the horizontal axis of the alignment view indicates the range of the spectrum view in the “Spectrum View Frame”. This frame will show you how the proposed ions align with the spectrum. By default, the “Spectrum Alignment Frame” displays b-ion and y-ion. The b ions are shown right to left in blue, while the y ions are shown left to right in red. Clicking on the “Survey” tab will display the corresponding precursor ion spectrum. The buttons that appear in this section are the same as those that are explained above in the “Spectrum View Frame” section. Click on the “Error Map” tab. The \( m/z \) ratio is displayed on the y axis and the error is listed on the x axis in Daltons. The “Error Plot” displays the confidence that is assigned to each ion. The most confident results lie on the centerline. Clicking a cell or column in the Ion Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.
**Peptide Details**
Click on the “Peptide Details” tab. The following window will appear:

At the top of the “Peptide Details” frame is the accession number of the protein that corresponds to the peptide that you chose in the “Peptide View” window. If more than one protein matches a single peptide, you will be able to select these additional proteins using the drop-down menu.

Below this you will see a simple alignment between the original de novo sequence for this spectrum (if available), the peptide found in the database and the reconstructed sequence. Letters on a green background, and with vertical bars, indicate agreement. *Color codes* on the de novo sequence letters still indicate positional confidence.

Finally at the bottom of the window you will see the sequence of the selected protein and in blue you will see where the selected peptide matches the protein. The darker the blue, the more confident the match. The matched peptides will be shown in red if you have performed a SPIDER search which is discussed in the next section.

**7.2.2 Protein View**
Click on the “Protein View” tab. The following window will appear:
The “Protein View” collects all the peptide identifications together, summarizes which proteins were present in the sample, and groups homologous proteins together. The same information is displayed in the Peptide View as in this Protein View; however, the results are organized to best enable us to evaluate at the protein level.

This view is helpful when building a summary that can be sent to a customer/collaborator. See chapter 13 for more details on exporting whole files or proteins of interest to an Excel file.

### Index

The top section of this view (shown above) behaves like an index, listing each protein found in the sample. Very similar proteins, containing the same set or a subset of the matched peptides, are clustered together. To expand and collapse the full list of proteins within each cluster click the ‘+’ or ‘-’ sign respectively.

The table below describes the contents of the columns in the index:

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>The GI, accession or other unique identifier for this protein as recorded in the database that was...</td>
<td></td>
</tr>
<tr>
<td><strong>Mass</strong></td>
<td>The calculated mass of this protein</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td><strong>Display</strong></td>
<td>A graphical coverage map. Blue areas represent parts of the sequence that have been explained by the identified peptides.</td>
</tr>
<tr>
<td><strong>Score (%)</strong></td>
<td>A value from 1 to 99 representing the confidence we have in this protein identification – calculated from the confidence on the ten best peptide hits for this protein, and normalized against the other identified proteins.</td>
</tr>
<tr>
<td><strong>Coverage (%)</strong></td>
<td>The number of amino acids in the protein sequence that have been explained by the identified peptides. Expressed as a percentage of the total length of the protein.</td>
</tr>
<tr>
<td><strong>Query Matched</strong></td>
<td>The number of spectra explained by matching to a peptide from this protein.</td>
</tr>
<tr>
<td><strong>Marked</strong></td>
<td>A multi-function checkbox. By default unchecked, but we can use this to select proteins for export or multiple sequence alignment.</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>The part of the protein’s header information as parsed from the database, usually it contains the name of the protein.</td>
</tr>
</tbody>
</table>

The columns themselves can be customized. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any of them to show or hide a column. These settings will apply to all your reports.

**Sequence Browser**

The “Sequence Browser” tab is selected by default. Clicking on a protein in the index will display the sequence of that protein below in the “Sequence Browser” panel. Clicking on the hyperlink of the accession number of the protein shown in blue will open a new window containing the webpage of the database that you searched for protein ID page in a new window.

There is also a “Peptides List” box which displays information about the peptides that matched to the selected protein. This list is identical to the “Peptide View” panel. Please see this section for more details.

Below the “Peptides List”, you will see the protein sequence with the matching peptide sequences in blue. The darker the blue, the more confident the match.
Click on the “Tool Box” tab:

- **NCBI BLAST Search**
  Search the selected protein sequence in the NCBI database.

- **NCBI Entrez Search**
  Search the selected protein name in Entrez across-database.

- **Multiple Sequence Alignment**
  Mark at least two proteins in the list and do multiple sequence alignment (MSA).

The selected protein will be displayed at the top of this page. Click on NCBI Blast Search or NCBI Entrez Search to search for the selected protein sequence in the NCBI database or the Entrez database, respectively.

To perform a multiple alignment search mark two or more proteins in the above list of proteins, by clicking in their checkboxes (see example below).

Then click on the “Multiple Sequence Alignment” link in the Toolbox window to generate the multiple sequence alignment in your web browser.

Identified peptides are highlighted in blue letters on the sequence. A more intense blue indicates a more confident match. The background colors indicate similarity between the sequences.
dark background indicates regions where residues or nucleotides are identical in all sequences, a light background indicates similarity across some sequences, and lowercase letters on white background highlight differences. A dash - is displayed where a gap had to be introduced in one sequence to complete the alignment.

<table>
<thead>
<tr>
<th></th>
<th>TRFE_BOVIN</th>
<th>ADH1_YEAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q29443</td>
<td>mrpyrallacavlgcladpertvwtciSchEanKcasF+ENv1RIesgpfsacvakkk</td>
<td>---------------</td>
</tr>
<tr>
<td>P00330</td>
<td>---------------</td>
<td>41DEtqKGV1yEShgKL</td>
</tr>
<tr>
<td>Q29443</td>
<td>tshnBciKsnSAdFV-1DqglVyeAGLknplLpvpvaEHtGk23qthyyavvvek</td>
<td>---------------</td>
</tr>
<tr>
<td>P00330</td>
<td>---------------</td>
<td>4xKd1--pVpKtXWnELIN--Vky5GvCHDLha--WxvswvplP</td>
</tr>
<tr>
<td>Q29443</td>
<td>kdtdfKLnElhKskch--tGLGrSa--Gnntpmaklykelpqgesig--IaanFfSaBCv</td>
<td>---------------</td>
</tr>
<tr>
<td>P00330</td>
<td>---------------</td>
<td>vKLplVcghEgagvV7SmGeNvkJNklq---------------dyglKe1NcSNC</td>
</tr>
<tr>
<td>Q29443</td>
<td>pCsdqyFwLyQqLcagkgtXDKcascHepyHES--GIF-KclmegAgdvAFkheAvuf</td>
<td>---------------</td>
</tr>
<tr>
<td>P00330</td>
<td>---------------</td>
<td>4xLEl--qNESIRG-MadLSXTHdS5KcQyataqAvqAHLIVv1Ad</td>
</tr>
<tr>
<td>Q29443</td>
<td>dnlpapdKnyeELLGdnTrksvdypecYLMVpsShAvtvugskedvHelnINHAcq</td>
<td>---------------</td>
</tr>
<tr>
<td>P00330</td>
<td>---------------</td>
<td>aqVa-------pHLaq1E--------VhKJlksAnMqgh---------Wve1SqaA</td>
</tr>
</tbody>
</table>
8. SPIDER Search

After having obtained de novo sequences for peptides that are not in the database, it is important to look for a homologous peptide in the database (Han et al., 2005). This will help you to learn more about the proteins in your sample. To search with SPIDER you must first have some good de novo sequences.

8.1 Setting up SPIDER Parameters

1) Select a data file or a Protein ID result from the “Project View” frame

2) Click the SPIDER icon on the toolbar

Or

Choose SPIDER Search from the Tools menu.

When a Protein ID results file is selected, the “SPIDER Search Options” window will appear as seen below:
If you have selected a data file, the following window will appear:

Note that this window differs from the other window as it asks you to select a database. It also does not give you any filter options.

In this case, we assume you already have de novo results for the data file.

Selecting a database, provides sequence variations for SPIDER to search against de novo sequences.

3) The following section will describe the different options that you have when setting up the parameters for your SPIDER search.

8.1.1 Query Options

Choose a Query Type. They are, in order of increasingly rigorous analysis:

Segment Match: this is not a true mutation search; instead, it will insist that the mass of the peptide returned is the same as that of the de novo sequence.

Non-gapped Homology Match: this search will allow for transpositions and single point mutations but not insertions or deletions.

Homology Match: this is the most rigorous (but most resource intensive) search mode, taking into account all types of mutations and the positional confidence scores. A quick version of this is used to create the reconstructed peptides and to generate the final scores in each of the previous search modes. This is the only search mode that allows you to use variable modifications.

8.1.2 General Options

Amino acid selection: Choose if you would like PEAKS to consider Leucine equal to Isoleucine without a penalty in the score as well as whether Lysine should be equal to Glutamine without penalty.
**Mass tolerance:** Enter the amount of error (in Daltons) that PEAKS will allow for when determining the peptide sequences.

**Number of peptides to report:** Choose how many of the best homologous peptides should be displayed after searching.

### 8.1.3 PTM Options

**PTM Options List:** The “PTM Options” list tells PEAKS what kind of post-translational modifications to include in its analysis. To view additional modifications, select the “Show unimod” box. If the desired PTM does not appear on the list or is different than what is listed, you can select the “New PTM” button and the “PTM Editing” window will open. Fill in the information pertaining to the PTM of interest. For a more in depth explanation of creating a new PTM, see section 7.1.4 PTM Options.

To select a PTM as Fixed or Variable, click within row of the desired PTM to highlight it, then click the button corresponding to its preferred designation, “Fixed Modification” or “Variable Modification”. If you incorrectly place a PTM in one of these sections, with it selected, simply select either Deselect, to have it removed from the list of selected modifications or Switch Type, to have it set as the other modification type. For example, using the Switch Type button, a PTM currently listed as fixed, will automatically be removed from the fixed modification list and enter the variable modification list, or vice versa.

Note that in previous versions of PEAKS, only fixed PTMs were allowed, however PEAKS version 5.1 allows variable PTMs as well when using the homology search.

**Max variable PTMs:** To reduce uncertainty, PEAKS' de novo sequencing 'vocabulary' can be limited by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

### 8.1.4 Filter Options

As the SPIDER search is computationally intensive, it is not recommended that you run all of your de novo sequencing peptides against the database; only those that cannot be well explained.

**de novo score (A.A.) threshold:** The SPIDER search requires a good sequence tag from de novo to be able to find good quality homologous proteins. Enter a value for the de novo score threshold. The recommended threshold is 0.5.

**Peptide score threshold:** Because there is no need to run SPIDER on peptides that already were found to have a good match during PEAKS protein ID, it is helpful to enter a peptide score threshold so that SPIDER will only be performed on peptides below the threshold. The recommended threshold is 0.65.
8.1.5 de novo Options

Because SPIDER requires a de novo sequence to find homologous proteins in the database, de novo sequencing will need to be performed first. If you have already done de novo sequencing, select the “I have already run de novo” button.

8.1.6 Database Options

Note that these options are only visible if you choose to run a SPIDER search on a data file rather than a PEAKS results file.

Database to search: Select from this drop-down list, one of the FASTA databases configured in PEAKS. To edit an already existing database, click on the “Edit Database” button. If the desired database is not in this list, click the "New Database" button. Note that you can also set up a new database in the “Database Configuration” window. The configuration window is the only place that you can delete databases that you have created. For more information on setting up new databases see section 15.2.3 Database Configuration.

Taxonomy selection: This list displays the taxa you have chosen for your search. If the database selected has taxon information available, you can click on the “Set taxa” button. Otherwise, the whole database will be searched. The selections correspond to established hierarchy -- i.e. selecting 'Mammalia' will search all of 'horse, cow, rat, mouse, human, etc.

4) After setting up parameters, we can save them for future use. Click the "Save Parameters" button and choose a name for future reference when prompted. Any parameters that you save will be available in the drop-down list at the top of the window. To see what's inside, just select one, and the parameters boxes will be populated.

5) Press the “OK” button and the SPIDER search will begin.

8.2 SPIDER Results View

SPIDER will search the database for homologous peptides and attempt to consolidate these into protein hits as well. The result report will look much like the results for PEAKS Protein ID or inChorus searching.

Clicking on the “Peptide View” tab will display results that look very much like the results for PEAKS Protein ID. See section 7.2.1 Peptide View for more details. Click on the “Peptide details” tab to see the SPIDER matches shown in red.

Letters on a green background with vertical bars, indicate agreement. Letters on a red background indicate sequencing error. Color codes on the de novo sequence letters still indicate positional confidence. Letters on a blue background indicate uncertainty or mutation. “+” signs represent more likely mutations. [Square brackets] indicate an equal
mass substitution, common non-critical *de novo* errors. <Angle brackets> indicate an equal mass substitution and a mutation.

When simply identifying exact peptides from the database using PEAKS Protein ID or inChorus, there is no need to reconstruct the ‘real’ sequence.

Clicking on “Protein View” will again yield a similar display as was seen for PEAKS Protein ID (see section 7.2.2 Protein View). In the “Display” column, red regions indicate areas of homology and potential mutation identified by SPIDER. Blue regions indicate areas of homology from the protein ID search, and purple indicates that the spectrum was identified by both SPIDER and protein ID.

Click on the “Coverage” tab. The highlighted colors are the same as those shown in the “Display” column above.
Chapter 9

9. PTM Finder

9.1 Setting up PTM Finder Parameters

1) Select a Protein ID results file to perform a PTM finder search on. Note that you cannot perform protein ID on a raw file or *de novo* results.

2) Click the PTM icon on the toolbar

*Or*

Select “PTM Finder” from the Tools menu.

The “PTM Finder Options” window will appear:

The parameters are the same as you used when performing protein ID (7.1 Setting Up Protein Identification Parameters) with the exception of the filter options found at the bottom of the window. As PTM Finder searches tend to be computationally intensive, PEAKS will only look at *de novo* sequencing results that are above the amino acid score threshold and below the peptide score threshold that you input.

*de novo score (A.A.) threshold:* The PTM finder requires a good sequence tag from *de novo* to be able to find good quality homologous proteins.
Enter a value for the *de novo* score threshold. The recommended threshold is 0.5.

*Peptide score threshold*: Because there is no need to run the PTM finder on peptides that were already found to have a good match during PEAKS protein ID, it is helpful to enter a peptide score threshold so that SPIDER will only be performed on peptides below the threshold. The recommended threshold is 0.65.

### 9.2 PTM Finder Results View

The results from a PTM finder search are identical to those seen in a PEAKS Protein ID search. Please see section 7.2 *Protein Identification Results* for more information on the PEAKS Protein ID search results.
10. *inChorus Meta Search*

inChorus protein identification can call upon several search engines (PEAKS protein ID, X!Tandem, OMSSA, Mascot and Sequest) for protein identification and will then compare and summarize the results from the different search engines in one single report.

Please note that if you wish to launch either Mascot or Sequest for an *inChorus* search, these engines are not internally provided with PEAKS and may need to be purchased by the respective vendors; however, licenses of these engines are not required if the result files have already been obtained. To set up your search engine preferences, see section 15.1.3 *Search Engine Preferences*.

**10.1 Setting up *inChorus* Parameters**

1) Select the orbisample.mzxml file

2) Click the “*inChorus Search*” icon on the toolbar

Or

Select “*inChorus Search*” from the Tools menu.

The “*inChorus Options*” window will appear:

3) First select each of the protein identification tools that you would like to use by putting a checkmark in their respective checkboxes. Search parameters for each program can be set by selecting the name of the search engine.

The option screens for each of the search engines available to *inChorus* are designed to work in the same way as options screens from the original programs. For help in setting search
parameters for each program, please refer to that program’s user manual. For help with PEAKS Protein ID, please see section 7.1 Setting up Protein Identification Parameters.

10.1.1 Importing Existing Results

PEAKS inChorus reads X!Tandem .xml files, OMSSA .omx files, Mascot .dat files and Sequest .srf files. When importing third party results files, please make sure that the scan number model in the results file is consistent with the one in PEAKS. PEAKS uses original data information to compute the inChorus score. When you run an X!Tandem search with command line, you need to turn on the option of “-w” in order to export data information into X!Tandem .xml files.

To import existing results, check the “Import Result” checkbox and select “Import Result”. The following window will open.

Although it is not necessary for the various search engines to use the same database in an inChorus search, it is necessary to have a unified database for an inChorus search that includes imported results. Select the database that you would like to use from the drop-down list. The inChorus search will be performed on all species in the database unless specified by the user. If this database does not appear in this list, refer to 15.2.3 Database Configuration, to configure your databases. To specify which taxa you would like to search, click on the “Set Taxa” button.

To import your file, click the “Browse” button that is found beside the appropriate search engine. Find the file that you would like to import and click “Open”. Once you have selected the file(s)
that you would like to import and have selected the options for any other search engine searches you would like to perform, click “OK”.

10.2 inChorus Results View
When the inChorus search is complete the “Project View” panel should contain a separate results file for each search engine that you selected as well as an inChorus report that combines the results from the multiple search engines. See an example below:

- PEAKS 3 [12-Jan-09 14:25]
- XTANDEM 4 [12-Jan-09 14:26]
- OMSSA 5 [12-Jan-09 14:23]
- INCHORUS 6 [12-Jan-09 14:28]

10.2.1 de novo, Peptide and Protein Views
Each results file for the 3rd party search engines looks very similar to the PEAKS protein ID results file (7.2 Protein Identification Results) with a few small differences. Firstly, there is no “de novo View” or “Chart View”, and secondly, the scoring will be specific to that search engine. For example, the score for OMSSA is listed as an E-value. For more information about different scoring methods, refer to the user manual of the 3rd party search engines.

The inChorus search report also looks very similar to the 3rd party search engine results; however there are no “de novo View” results available. The de novo sequencing results that are found in the “de novo View” are only those that correspond to results that have been identified by one of the search engines in the inChorus search.
11.  Creating a High-Throughput Work Flow

In some situations, you may have many data sets that you wish to process all at once, with the same parameters. PEAKS 5.1 allows you to do this type of work with minimal effort on your part. By setting up a work flow, you can start a batch process of several data files and not worry about it until it is finished.

*It is important to note that all the files you load will be processed in exactly the same way, using exactly the same parameters. If you want to do some differently than others, we must set up another work flow.*

11.1 Setting up the Work Flow Parameters

Click the “Work Flow” icon on the toolbar.

The “Work Flow Configuration” window will appear:

The steps will appear as “unfinished” until they have been completed, at which time they will be labeled “finished”.

Click the “Select Data” button. The following window will open:
Only projects that are open in the “Project View panel” can be selected for analysis with the work flow function. To select which files/samples you would like to analyze, select the individual file/sample or click the “All Samples” or “All Fractions” button and then click the “Add to Right” button to transfer the samples/files to the “Selected Data” panel on the right hand side. Use the “Remove” and “Clear” buttons to remove selected files/samples or all files/samples, respectively from the “Selected Data” panel. Click “OK” to move to the next step.

Check the boxes beside the functions that you would like to perform with the work flow. Note that PEAKS requires de novo sequencing results to perform Protein ID. The parameter windows that open when you select the various functions in the work flow are identical to those that appear when you perform each function individually. Please refer to the chapters on each individual function if you require more details on setting up the parameters.
12. Filtering Your Results

PEAKS 5.1 provides you with an exhaustive list of all proteins and peptides that can be found in a sample. However, as many researchers have their own criteria of what information is required in their report and what is an acceptable result, PEAKS 5.1 provides the necessary filtering tools that enable filtering out the less critical information, leaving just the essentials.

12.1 Setting Filter Parameters

Click on the time and date stamp associated with the result that you would like the filter. Once the report loads, click the right button on your mouse and select “Perform Filtering”.

The following window should appear:
12.1.1 Possible Filters/ Selected Filters/ Edit Filter

The filters are grouped into three basic types to reflect what they act on:

- *de novo* filters act to remove proposed *de novo* sequences
- Peptide Filters act to remove peptides found in the database from the report
- Protein filters act to remove proteins from the report

To see the available filters for each level of filtering, double click on the appropriate folder in the “Possible Filters” frame. See the options in each folder below:

Choose a filter from the ‘Possible Filters’ list on the left by clicking on it. Options for this filter will appear in the “Edit Filter” frame. Once you have set the options that you would like for the filter in the “Edit Filter” frame, drag the filter that you would like into the “Selected Filter” list on the right hand side. Click “OK” to apply the filter that you have selected to the current file.

If you would like to add another filter, you can repeat the process, continuing to add as many filters as necessary. In this way it is also possible to have two filters on the same property; we can set a range of protein mass, for instance, by applying one filter on the upper bound of the mass and adding another filter to be the lower bound of the mass. We can also have more complex filters that involve multiple properties.
For example, let’s say that you want to show only proteins with more than one high scoring (greater than 60% score) peptide, a standard requirement for publication. Double click on the “Peptide Filters” folder. Select “Score Filter”. Edit the filter to select peptides that have a score that is greater than 60% in the “Edit filter” frame.

Now drag “Score filter” from the “Possible Filters” frame to the “Selected Filters” frame.

### 12.1.2 Filter Options
The filters cascade through each view in the multi-part report. For instance, removing a peptide from the database search results – the Peptide View list – will cause the de novo sequence for that peptide to be removed from the “de novo View” tab but will not affect the “Protein View” list. Filtering out a protein (by mass, for example) will remove it from the “Protein View” list and will remove all peptides associated with that protein from the “Peptide View” list as well as from the “de novo View” list. The manner in which the “de novo View” is linked can be specified by the user using the options in the “Filter Options” frame. See the figure below for more information.

Filtering out (removing) a de novo result does not affect what is displayed in the Peptide View tab or Protein View tab. Filtering out (removing) a de novo result also removes that spectrum from consideration in the Peptide View tab and Protein View tab. Again, filtering out (removing) a de novo result also removes that spectrum from consideration in the Peptide View tab and Protein View tab.
12.1.3 Parameter Options

Filter sets can be saved and re-used between sessions by clicking the “Save Parameter” button that is found at the top right hand corner of the “Filter Parameters” window.

You may prepare your results the same way each time; in which case it makes sense to set up a filter that will be automatically applied each time we load a report. Select a filter from the list of saved filters from the drop-down menu found at the top right hand corner of the “Filter Parameters” window. Click on the “Set saved as default” button. This filter will be displayed in the “Parameter Options” frame (as seen below) and will be applied automatically just after a report is loaded. Be careful, if your default filter is very stringent, it can sometimes remove everything! To remove a default filter, press the Clear Default Button at the bottom of the Filter Pane.

Each filter can be applied several times over. So it can get a little complex. To illustrate, here are a few examples:

1) Goal: Show proteins that have two high-scoring hits:
   - Add the Protein Filter called “Query” and in the “Edit Filter” section choose ‘greater than’ and type ‘1’ in the box (without the quotes). This will remove any ‘one hit wonders’.
   - Add the Peptide Filter called ‘Score’ and in the “Edit Filter” section choose ‘greater than’ and type ‘50’ in the box (without the quotes).

2) Goal: Find a protein that contains the word ‘human’ or ‘rat’ in the database entry’s description, but not Keratin or Trypsin.
   - Add the Protein Filters called ‘Desc’
   - In the “Edit filter” section, you are required to type in a regular expression (regex). This allows you to use wildcards.

<table>
<thead>
<tr>
<th>Wildcard</th>
<th>Meaning</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>.*</td>
<td>“Anything of any length”</td>
<td>.<em>human.</em> Will find anything that contains the word ‘human’, with anything before and anything after.</td>
</tr>
<tr>
<td></td>
<td>“Or” (use brackets)</td>
<td>*(human</td>
</tr>
<tr>
<td>?!</td>
<td>“Not” (use brackets)</td>
<td>(?!(Keratin</td>
</tr>
<tr>
<td>[]</td>
<td>“Any of these characters”</td>
<td><em>(Hh)[uman] (Rr)[at].</em> Will find anything containing the words Human, human, Rat or rat.</td>
</tr>
</tbody>
</table>
• Type in the regex: (?!.*(Keratin|Trypsin).)*(human|rat).* and press the Enter key. If PEAKS confirms that this is a valid regular expression, it will put a check in the ‘Valid Java Regex’ box.

3) Goal: Setting a protein mass range
If we know the approximate mass of the proteins you are interested in, you can eliminate all proteins that are not close in mass.
  • Add two filters: “Protein Filters: Mass >12000” and “Protein Filters: Mass < 32000”.
13. Complex Analysis

13.1 Creating a project for complex system

PEAKS 5.1 is able to analysis MS data from very complex systems. The data analysis scheme is organized as follows:

- Project nodes
- Sample nodes
- File nodes

Below is an example of a project that contains three samples that were generated using the following fragmentation methods: ETD, CID, and CID/ETD. Each sample has three files.

13.2 Integrating data analysis

Within a project, the data is analyzed either file by file or sample by sample. By selecting a sample, the operation applies to all files in the sample. It means all spectra from different files are processed in a single run. The result node is displayed at same level as selected data node.

PEAKS 1 is the PEAKS Protein ID result for file ETD_01.RAW, whereas PEAKS 11 is the PEAKS Protein ID result for all three fractions of the ETD sample. Note that the result of a sample may not be the sum of the results of all files in the sample.
14. Exporting Data/Reports and Printing

PEAKS 5.1 allows you to create reports to share with collaborators, colleagues and clients. The reports are available in HTML or Microsoft Excel (.xls) formats and follows a ‘What You See Is What You Get’ philosophy. All the information you see on screen in PEAKS 5.1 will appear in the exported report. For this reason, it is important that we complete results filtering and toggling columns before exporting a report.

14.1 Export Data in .mzxml or .mgf
In order to export your data file in .mzxml or .mgf right click on the data file that you wish to export.

Click “Export MGF File” A window will open that will prompt you to enter a name and a location for the file. Click “Export”.

or

Click “Export MzXML File”. The following window will open:

![Export MzXML File](image)

Enter the starting and ending retention times in the appropriate boxes. Then click the “Browse” button to select a destination to save your file.

14.2 Export Results File
To export the results for a selected data file, right click on the data file and select “Export Result file”. All of the PEAKS results file generated for the selected data file will be displayed in Excel format.
14.3 Export Peptide Results in PepXML Format
In order to export your PEAKS results file in PepXML right click on the results file that you wish to export and select “Export PepXML”.

The following window will appear:

Browse through your computer to select the location that you would like to place the exported PepXML file. Then click “OK”.

14.4 Export Results in Excel Format
In order to export your PEAKS results file to Excel right click on the results file that you wish to export and select “Export Excel”.

The following window will appear:

If you would like to export all of the protein and peptide results select the “All Protein and Peptide Result(s)”, otherwise select one of the other options where you can limit which results are exported. Select the appropriate boxes if you would like to export “Data Properties”, “Search Parameters” and “Filter Conditions” to Excel. Finally you need to select the “Export Destination” by clicking the “Browse” button. Then click “OK”.
If you export *de novo* results to Excel, the *de novo* sequencing results will be exported and you have the choice to also export “Data Properties”, “Search Parameters” and “Filter Conditions”. See below:

### 14.5 Print Tables and Graphs for Publication

In order to export an image file, right click on the results file that contains the appropriate image file and select “Export Statistics Graph”.

#### 14.5.1 *de novo* Image Files

Select a *de novo* results file. Right click and select “Export Statistics Graph”. The following window will appear:
The “de novo ALC Score Chart” option will be selected in the “Statistics Graph Type” panel. Select a file format and height/width for your chart and browse your computer to select a destination. Beside is an example of the output:

### 14.5.2 Protein ID Image Files

Select a Protein ID results file. Right click and select “Export Statistics Graph”. The following window will appear, giving you the option of exporting a “Protein Score Chart”, a “Peptide Score Chart” or a “False Positive Rate Chart”: 

![Export Statistics Graph Specification](image)
Below are examples of the “Protein Score Chart”, the “Peptide Score Chart” and the “False Positive Rate Chart”, respectively.
15. Advanced Configuration and Environment Preferences

15.1 PEAKS Environment Preferences

This section will describe the setting up the configuration of environmental preferences including general, instrument, search engine and ion editor configurations.

To begin click the Preferences toolbar icon
Or
Select “Preferences” from the “Window” menu.

The following window will open:

Use the ‘+’ and ‘-’ boxes to expand and collapse the view.

15.1.1 General Preferences

*Default Input File Directory*- Select where your data is being inputted from using the “Browse” button.

*Default Output File Directory*- PEAKS outputs your results to C:\derbyServer by default. Select the “Browse” button to change this location.

*Project folder*- PEAKS uses C:\derbyServer\serverDB as the default project folder. Select the “Browse” button to change this location.

*Temporary File Directory*- PEAKS uses C:\temp_mgf as the default project folder. Select the “Browse” button to change this location.
**Default Configuration File Directory** - Your configuration files for PEAKS can be found at C:\Documents and Settings\bsi\peaks\peaksconf.xml by default. Select the “Browse” button to change this location.

**Default Log File Location** - Your log file for PEAKS can be found at C:\Documents and Settings\bsi\peaks\peaks.log by default. Select the “Browse” button to change this location.

**RMI Connections**
Clicking on “RMI connections” on the menu on the left hand will open the following window:

![RMI Connections Window](image)

The default port numbers for the Server, Client and Worker will appear. The port numbers can be changed if conflicts arise. Contact technical support at BSI for more information.

Click the “Apply” button to save any changes that you made.

**Derby Database**
Clicking on “Derby Database” on the menu on the left hand will open the following window:

![Derby Database Window](image)

**Derby Host**
The name of the “Derby Host” as well as the “Port” number will come up by default and can be changed if needed.

**Memory used to start Derby Server**
The amount of “Memory used to start Derby Server” will also come up by default but can be changed if more memory is available.

**Derby Jar Location**
The “Derby Jar Location” panel will list the location of the Derby Jar file by default. If you would like change this location, check the “User Define” box and click on the “Browse” button to select a new location.

Click the “Apply” button to save any changes you have made.

**Performance**
Clicking on “Performance” on the menu on the left hand will open the following window:

![Preferences Window](image)

**Computer Performance**
Select the number of cores that your computer contains (i.e. single, double or quad core).

**3D View**
PEAKS will display a 3D view with your quantification results. Check the “Show 3D View” box to enable this function. PEAKS 5.1 comes with the Java3D program to support the viewing of 3D images.

**Fast loading files**
This function is for raw file loading. If you check the “Fast Loading Files” box, PEAKS will only load spectrum header information without the peak list. Please note that if you set up a project which contains more than 20 raw files, this function will not work well due to memory issues, and you should uncheck the “Fast Loading Files” box.

Click the “Apply” button to save any changes you have made.

**15.1.2 Instrument Preferences**
This section will allow you to change any preferences for the following instruments: ABI, Bruker, Shimadzu and Varian.

**ABI (.wiff)**
Clicking on “Instrument” and then “ABI (.wiff)” on the menu on the left hand side will open the following window:

**Default .wiff raw file convertor location**
Click “Browse” to tell PEAKS the location of the Default .wiff raw file converter.

**Raw file convertor options**
ABI raw files may contain several samples. By default, these samples are not merged into one data set. Select “yes” if you would like PEAKS to merge all the samples into one data set.

For PEAKS to work optimally, it is important to select if the survey spectrum or the product spectrum has been centroided.

Click the “Apply” button to save any changes you have made.

**Bruker (.yep/baf, fid)**
Clicking on “Instrument” and then “Bruker (.yep/baf, fid)” in the menu on the left hand side will open the following window:

**Default compass file location**
Click “Browse” to tell PEAKS the location of the CompassXport file converter.

**Raw file convertor options**
Bruker .fid files may contain several samples. By default, these samples are not merged into one data set. Select “yes” if you would like PEAKS to merge all the samples into one data set.

Click the “Apply” button to save any changes you have made.

**Shimadzu AXIMA (.run)**
Clicking on “Instrument” and then “Shimadzu AXIMA (.run)” in the menu on the left hand side will open the following window:

Click “Browse” to tell PEAKS the location of the Shimadzu run2xml.exe file. Click the “Apply” button to save any changes you have made.

**Varian (.xms)**
Clicking on “Instrument” and then “Varian (.xms)” in the menu on the left hand side will open the following window:

Click “Browse” to tell PEAKS the location of the xmlrai.exe file. Click the “Apply” button to save any changes you have made.

**15.1.3 Search Engine Preferences**

**X!Tandem Settings**
Clicking on “Search Engine” and then “X!Tandem Settings” in the menu on the left hand will open the following window:

Start by selecting whether you would like PEAKS to access a server or local version of X!Tandem. If you select the server version, you must enter the Host name (or IP address) as well as the port. To make sure that you entered everything correctly and that the server is working, click the “Test Connection” button.

As PEAKS provides a local copy of X!Tandem, upon installation, a default path will appear in the Local Settings section. If you wish to use another license/location for X!Tandem, click the “Browse” button to tell PEAKS where to find the search engine. Click the “Apply” button to save any changes.

OMSSA Settings
Clicking on “Search Engine” and then “OMSSA Settings” in the menu on the left hand will open the following window:

As PEAKS provides a local copy, upon installation, a default path will appear here. If you wish to use another license/location for OMSSA, click the “Browse” button to tell PEAKS where to find the desired path. Click the “Apply” button to save any changes you have made.

Sequest Settings
Clicking on “Search Engine” and then “Sequest Settings” in the menu on the left hand will open the following window:
To use Sequest click the “Browse” button to tell PEAKS where to find the search engine. You must also browse your computer to find the location of the “Default Sequest Parameter File (.params)” as well as the “Sequest Result Output Folder”. Click the “Apply” button to save any changes you have made.

**Mascot Settings**
Clicking on “Search Engine” and then “Mascot Settings” on the menu on the left hand side will open the following window:

In this window you will tell PEAKS how to access your Mascot server (if applicable). Enter the Host name (or an IP address), Port, Virtual Directory as well as your user name, password and email address. To make sure that you entered everything correctly and that the server is working, click the “Test Connection” button. If you would like to save your password so that you don’t have to enter it every time, check the “Save Password” box. Click the “Apply” button to save any changes you have made.

### 15.1.4 Ion Editor Preferences
Clicking on “Ion Editor” on the menu on the left hand side will open the following window:

**Decimal places**
Select the number of decimal places you would like to appear in the ion table. The default is set to two decimal places.
**Ion Editor**

Clicking on “Ion Settings” and then “Ion Editor” in the menu on the left hand will open the following window:

To select an ion type to be viewed in the ion table, click on the ion type in the “Ion Type” list found on the left hand side of the window. You now need to select the charge for that ion type from the drop-down menu. Once you have done this, click on the “=>Add with charge” button and the ion type will now appear in the “Ion Table Columns” list on the right hand side of the window.

To remove an ion type from the “Ion Table Column” list, select the ion type and click on the “Remove from list <=” button. The ion type will now appear in the “Ion types” list.

Click the “Apply” button to save any changes you have made.

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### 15.2 PEAKS Configuration

This step includes configuration of enzymes, PTMs, databases, instruments, and parameters.

To begin click the Configuration toolbar icon.

Or

Select “Configuration” from the “Windows” menu.

#### 15.2.1 Enzyme Configuration

PEAKS can use almost any enzyme, or combination of enzymes in your analysis. You can select from any of the built-in enzymes or define your own. From the “Configuration” window select “Enzyme” from the left hand to change your enzyme configuration.

**Built-in enzymes**

All of the built-in enzymes within PEAKS are listed in the “Enzyme list”. Clicking on one of these built-in enzymes will display the information listed about that enzyme in the “Enzyme Details” panel. Note that you cannot delete or change the details of a built-in enzyme and therefore the “Delete enzyme” button and the “Digest Rules” panel will be grayed out.

**Create a new enzyme**

Click on the “New Enzyme” button.
Digest Rules: This is how you specify where you enzyme will cleave the protein between two amino acids to create peptides. The letter X denotes ‘any amino acid in this position’, while {set brackets} indicate any amino acid except the one in the brackets. You can also choose to select the check the box “Select peptides that satisfy the above rules at both ends” if you desire.

The example below shows a combination of Trypsin and Asp-N.

You must click the “Add/Update button for the changes to be saved. Your new enzyme will now appear in the “Enzyme List” where you can access it later. If you wish to delete an enzyme that you created, select the appropriate enzyme and click the “Delete Enzyme” button.

Note: For information on defining new enzymes “on the fly” for PEAKS de novo or PEAKS Protein ID, see section 6.1.2 Enzyme Options or 7.1.2 Enzyme Options, respectively.

15.2.2 PTM Configuration
From the “Configuration” window select “PTM” from the left hand to change your PTM configuration.

Built-in PTMs
The built-in PTMs within PEAKS are listed in the “PTM List”. To see additional built-in PTMs from the Unimod library, click the “Show unimod” box. Clicking on one of these built-in PTMs will display the information listed about that PTMs in the “PTM Details” panel. Note that you cannot delete or change the details of a built-in PTM and therefore the “Delete PTM” button and the “PTM Rules” panel will be grayed out.

Create a new PTM
Click on the “New PTM” button”. Now simply enter the information about your PTM in the “PTM Details” panel.

Name: this name will appear in the PTM list for future use after it is saved.

Monoisotopic mass: the mass that the residue gains or losses as a result of the PTM.
Neutral loss mass: the mass that the modified residue loses as a result of fragmentation. Ex. 28 would signify a loss of 28 Daltons.

Chemical formula: the chemical formula of the PTM. This should correspond to the mass listed above.

Residues that can be modified: Enter residues that can be modified anywhere, residues that can only be modified if they are at the N-or C-terminus or in the middle only.

Rule: you can enter a comment for your reference.

You must click the “Add/Update button for the changes to be saved. Your new PTM will now appear in the “PTM List” where you can access it later. If you wish to delete a PTM that you created, select the appropriate PTM and click the “Delete PTM” button.

The example listed below is one where we knew that only methionine was oxidized.

Note: For information on defining new PTMs “on the fly” for PEAKS de novo or PEAKS Protein ID, see section 6.1.3 PTM Options or 7.1.4 PTM Options, respectively.

15.2.3 Database Configuration

In addition to de novo sequencing of peptides, PEAKS 5.1 also has the ability to search through a database search to identify proteins. In order to use this function, PEAKS must have access to a protein or EST database in FASTA format or an EST database of DNA sequences. You can point PEAKS to an existing database on your system, or download one. Additionally, you can associate taxonomy with certain databases.

From the “Configuration” window select “Database” from the left hand to change your database configuration. The “Database list” at the top of the screen will show you databases that you have already configured. Select one of these files to see the details in the “Database Details” panel below.

Configure a new database

1) Select the “New Database” button on the right hand side of the “Database List”. You will now be filling in the specifics for your database in the “Database Details” panel below.
2) **FASTA Format Database**: Select your database from this drop-down menu, or select “Other” if your database is not in the list or if you would like to submit your own database.

3) In the basics option panel, enter a name for your database and select “Download Database”. The following window will appear:
4) If you would like to invoke your default FTP client software and download automatically, click “Yes”. If you select “No” the following window will appear telling you that the URL will be copied to your system clipboard. Click “Ok”.

Open your Internet Explorer and paste the URL into the address bar. A file download window will open. Click Save.

5) Once the database is downloaded, you need to make sure that you decompress the file if it is compressed using a program such as WinZip, or WinRar to extract its contents. The file inside the compressed file will be a FASTA format text file (a .fas or a .fasta file).

6) Finally put the database file into a directory that PEAKS can access.

7) Click “Browse” to tell PEAKS where the database file is located.

8) If the database that you have selected is an EST database, check the box labeled “EST Database”. If not, leave it blank.

9) Since you have already selected a “FASTA Format Database” in Step 2, the Accession number information and the parsing rules for the database headers are shown in the textboxes below in the “Advanced Options- Fasta Title Format” panel.

If you chose “Other” (in step 2) you must enter parsing parameters by completing the textboxes. Alternatively, if the database format is the same as one of the public databases, you can choose to apply that database’s format when PEAKS reads the database. Select the database that is similar to yours from the drop-down list to fill the textboxes with the appropriate parsing rules.

A note on parsing rules
Apart from starting with a “greater than” symbol, the precise syntax of the FASTA title line varies from database to database. For this reason, PEAKS uses Java Regular Expressions to
define how the accession string and the description text should be parsed from the FASTA title line.

**A note on using a delimiter**
Some databases use one entry to represent multiple protein entries. The FASTA headers are concatenated with a delimiter. Since some of these databases use unprintable control codes as delimiters PEAKS will use the equivalent ASCII decimal code to represent them. For example the NCBI NR database uses CTRL-A as a delimiter so the user should input "1" as its equivalent decimal delimiter as listed here.

10) To be able to do PEAKS Protein ID using a specific taxonomy, you will need to download some files and place tell PEAKS where to find them in the “Taxonomy Options” panel.

11) To download the taxonid file, click the “Download” button. The following window will appear:
If you would like to invoke your default FTP client software and download automatically, click “Yes”. If you select “No” the following window will appear telling you that the URL will be copied to your system clipboard. Click “Ok”.

Open your Internet Explorer and paste the URL into the address bar. A file download window will open. Click Save. Repeat Step 11 taxdmp file. Please note that you do not have to decompress the taxonomy files.

12) Now that you have downloaded the taxonomy files, you must tell PEAKS where to access them by clicking the Browse button and selecting the file.

13) To save the database to your “Database List”, you must click the “Add/Update” button before clicking the “Ok” button.

**Delete a previously saved database**
If you would like to delete a database file, select the database that you wish to delete and click on the “Delete Database” button.

**Set a database as default**
Select the file and click the set as default button which is located to the right of the “Database List”. This database will now be used by PEAKS when you run PEAKS Protein ID.
Moving/Updating a Database
If you choose to move a database to another directory or delete it entirely, you need to notify PEAKS. You must remove the database from the list and re-load it. Until you do so, the database name will appear in red in the list of databases and any protein identification using that database will fail.

If you choose to update the database (perhaps by downloading the latest database file and overwriting the old database file), PEAKS will show the database information in light gray. A light grey color could also mean that the database does not have header information.

Best practices: configuring databases for use with X!Tandem
At the time of this writing, X!Tandem had trouble searching through large databases, and would crash. It is therefore suggested that X!Tandem only be used with small databases; or if used with a large database, a taxon should be specified. The NCBI nr and SwissProt databases are ideal for this purpose.

Best practices: configuring databases for use with OMSSA
At the time of this writing, we could not use OMSSA with databases that were not in NCBI format, or SwissProt format, and have those results available to inChorus. Also, a bug in OMSSA prevents us from easily using databases with OMSSA when they are stored in a folder that contains a space in its path. This creates problems when PEAKS creates temporary databases on our behalf. To avoid this, best practices suggest that you put all our databases in a folder “c:\peaksdatabases”. The folder “c:\my documents\databases” wouldn’t work because it contains a space between ‘my’ and ‘documents’. Using spaces in the database file name causes the same problem. So after you download and extract our database you should call the database file “ncbinr.fas”, or “ncbi_nr.fas” rather than “ncbi nr.fas”.

15.2.4 Instrument Configuration
From the “Configuration” window select “Instrument” from the left hand to change your instrument configuration.

Built-in Instruments
Select the manufacturer of your instrument from the drop-down list. The names of the instruments will then appear in their vendor specific formats. Select your instrument and you will be able to view the information on your instrument in the “Instrument details” panel below. You can also select “General” in the manufacturer list and the instruments will be listed in a general format.

Note that you cannot delete or change the details of a built-in instrument and therefore the “Delete PTM” button and the “Instrument Details” panel will be grayed out.

Create a new instrument
Click on the “New Instrument” button and the following window will appear:
In the “Instrument Details” panel, create a name for your instrument.

Next, fill in your details in the “Basic Options” panel. In the manufacturer drop-down list, select a specific vendor or “General”.

**Ion Source**: Use the drop-down list to select what ion source that was used; MALDI/SELDI or ESI(nano-spray). This will help the PEAKS Data Refine tool to decide the charge of the ions.

**MS- Precursor Scan**: Use the drop-down list to select what type of MS scan was performed. This selection will tell the PEAKS Data Refine tool if the survey scan is of sufficient resolution to determine the charge and the monoisotopic peak from the examination of the survey scan.

**Fragmentation type**: Use the drop-down list to select the method of fragmentation that was used. This selection will tell PEAKS what type of ion-series to expect for PEAKS auto *de novo* sequencing and PEAKS protein ID database search. Select CID/ECD if alternating fragmentation is used to allow the algorithm to determine the type of fragmentation from each scan header.

**MS^n Product Scan**: Use the drop-down list to select what type of MS^n scan was performed. This selection will help PEAKS decide which internal parameters (for weighing fragments and amount of noise) to use during PEAKS auto *de novo* sequencing and PEAKS protein ID database search. Select LIT/FT if alternating hi-res/low-res modes are used, allowing the algorithm to determine the mass analyzer from the scan header. You can also use the “Advanced Options” to specify additional parameters.

**Precursor Mass Search Type**: Select “Monoisotopic” or “Average.” For ion-trap instruments, it is usually beneficial to allow the PEAKS protein ID database search to use an average mass.

**Parent and Fragment error mass tolerance**: User specified values. These will appear on the PEAKS *de novo* and PEAKS protein ID options screens when the instrument is selected.

**Target Ions**: Select which ions that you would like PEAKS *de novo* and Protein ID to focus their search on.
You must click the “Add/Update button for the changes to be saved. Your new instrument will now appear in the “Instrument List” where you can access it later. If you wish to delete an instrument that you created, select the appropriate instrument and click the “Delete Instrument” button.

15.2.5 Parameter Configuration

From the “Configuration” window select “Parameters” from the left hand to change your parameter configurations. Please note that you can only view and delete parameters from within this parameter window. From the “Parameter type” drop-down list at the top of the screen you can select de novo, PEAKS Parameters or SPIDER Parameters. The parameters that you have saved within these categories will be displayed below in the list. Select the parameter file that you would like to view.

Creating a new parameter file

If you would like to create and save new parameters you can do this when/before you set up auto de novo sequencing (see section 6.1 Setting up Auto de novo Sequencing Parameters), PEAKS protein ID (see section 7.1 Setting up Protein Identification Parameters) or SPIDER (see section 8.1 Setting up SPIDER Parameters). These references will provide you with an explanation of all of the parameters.

Deleting a previously saved parameter file

If you would like to delete a parameter file, select the file that you wish to delete and click on the “Delete” button.

Viewing a previously saved parameter file

Selecting a file will display the details of that file below. For an explanation of the parameters, please see the pages listed in the “Creating a new parameter file” section above.
16. PEAKS Quantification Module

Many approaches to protein quantification using mass spectrometry data have been described in the literature. In terms of their implementation, most of them can be classified into three protocols.

- **MS:** Quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set. This is the most widely used approach, which can be used with any chemistry that creates a precursor mass shift. For example, ICAT and SILAC.

- **MS/MS:** Quantification based on the relative intensities of fragment peaks at fixed m/z values within an MS/MS spectrum. For example, iTRAQ and Tandem Mass Tags.

- **Label free:** Label free quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

All three protocols are fully implemented within PEAKS Q. The flow chart is shown below:

### 16.1 Setting up PEAKS Q Parameters

1) In the “Project View Frame”, select a PEAKS Search result file.
2) Click the PEAKS quantification toolbar icon.
   Or
   Select “Quantification” from the “Tools” menu.

The following window will open, displaying the quantification parameters:
Slightly different options will be available if you select labeling at the MS level:

Quantification parameter options include the following:

**Basic Options**

*Mass Error Tolerance:* Quantification is based on the feature of a peptide that identifies its origin in the sample mixture. For example, in a SILAC experiment, one feature is unmodified peptides and the other is peptides modified with Label:13C(6) on Arginine or Lysine. For iTRAQ, the feature would be reporter ion m/z value. The mass error tolerance is for pairing up features.
Upper Bound of Precursor Charge: The peptide may present in different charges. Upper bound of precursor charge defines the maximum charge of peptides which are used for counting quantity.

Retention Time Range: The retention time range is for pairing up features. For iTRAQ, it is optional.

Labeling Options

Labeling occurs at the MS/MS level e.g. ITRAQ: It is for quantification based on the relative intensities of fragment peaks at fixed m/z values within an MS/MS spectrum.

Labeling occurs at the M level e.g. ICAT: It is for quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set.

Label-free: It is for quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

Sample: It is for specifying sample name.

Reporter ion: It is for specifying mass of reporter ion

Added Mass: The modified mass of a residue.

Residues: The residue to be modified.

Labeling efficiency: It is for specifying efficiency of chemical reaction.

Add label: It is used to add a label.

Delete label: It is used to delete a label.

16.2 3D View
In order to produce a 3D view, you must first select this in your preferences.
1) Click on the Preferences toolbar icon.
   or select “Preferences” from the “Windows” menu.
2) Select “General” from the panel on the left hand side.
3) Select “Performance” and check the “Show 3D View” box.
4) Click “Apply”.

110
When a PEAKS Q run is complete, a “3D View” tab can be found in the “Peptide View” window:

The 3D View contains 3 axes: intensity, m/z ratio and scan number.

The panel along the bottom allows you to narrow in on the peptides that you would like to examine.

You can specify a particular scan number range, m/z range or intensity range.

Click the “Apply” button to change the 3D view to your specified values.

Check the “Auto” button and click “Apply” to the default numbers.

16.3 iTRAQ Walkthrough

Isobaric Tagging for Relative and Absolute Quantification (iTRAQ) uses isotopic labeling to enable relative quantitative comparisons. Up to eight different proteomic samples can be labeled using eight different isobaric tags.

16.3.1 Setting up a Database

Before running the walkthrough data, you need to set up a database. So that this can be a quick process, we have provided you with a sample fasta database called “SampleDB.fasta” in your PEAKS program folder (C:\PEAKS Studio 5.1\Data).

Click on the configuration toolbar icon or select “Configuration” from the “Tools” menu. Select “Database” from the left hand side of the window. Under “Database Details” enter the following information:

- **FASTA format database:** UniProtKB/Swiss-Prot
- **Database name:** Sample DB
- **Path:** C:\PEAKS Studio 5.1\Data\SampleDB.fasta

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111
16.3.2 Creating a Project

Click on the “Create new project” icon or select “New project” from the “File” menu. Give a name to your project, for example “iTRAQ Sample”. Click Add data files. Locate “iTRAQSample.mzxml” from C:\PeaksStudio5.1\Data.

With the file highlighted, select, Quad-TOF from the “Instrument Type” drop-down list.

Click “OK”.

Double click on the iTRAQ sample file.

Your “Main Processing Screen” should look something like this:
For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once. You will need to wait until the file has loaded (i.e. the file node looks like this: 🌞).

See section 16.2 3D View to enable 3D view.

16.3.3 Setting up the Workflow Parameters

Click on the workflow button “♫”. The following window will appear:

Click “Select Data”.
The following window will open:

Select the iTRAQSample.mzXML file and then click “Add to Right”. The samples should now appear in the “Selected Data” panel as shown below:

Click “OK”.

The workflow window should now display “finished” beside the “Selected Data” button.

Check the 2nd box and then click the “Refine data” button.
Enter the following parameters:

Click “OK”.
Check the 3rd box and then click the “de novo” button. Enter the following parameters:
Enter the PTMs as shown below by selecting “Set PTM”:

Click “OK”. Check the 4th box and then click the “PEAKS Search” button.
Enter the PTMs as shown below by selecting “Set PTM”:

Check the 5th box and then click the “Quantification” button. Select “Label at the MS/MS level” from the left hand panel and enter the following parameters:
Note: To add information into the “Label Options” panel, click the “Add Label” button to add a new sample.

Click “OK” and then click “Start” in the workflow configuration panel. The following window will open:

Click “Start Jobs”.

16.3.4 ITRAQ Results

Once completed, the protein quantification result will be displayed in the PEAKS Protein ID node. Double click on this node and the “Peptide View” tab will appear by default. The quantification results are listed as a “Ratio of 117.115:114.112”:

Click on the “Peptide Details” tab to see a simple alignment between the original de novo sequence, the peptide found in the database and the reconstructed sequence. At the bottom of the “Peptide Details” panel you will see where the selected peptide matches the protein highlighted in blue.
PEAKS provides the 3D View of each peptide feature for visual validation. Click on the 3D View tab. The panel along the bottom allows you to narrow in on the peptides that you would like to examine. You can specify a particular scan number range, m/z range or intensity range. Click the “Apply” button to change the 3D View to your specified values.

Select the “Protein View” tab. The quantification results are listed as a “Ratio of 117.115:114.112” and as “Standard Deviation of 117.115:114.112”. They are highlighted in the red box below. For example the relative protein ratio for the top ranked protein (Beta-galactosidase) is 3.01 with a standard derivation of 0.45:

16.4 SILAC Walkthrough

Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) is a method to metabolically label proteins for relative quantitative comparison. One cell population is fed amino acids of normal isotopic composition; the other cell population is fed amino acids labeled with heavier isotopes. The heavy amino acids are incorporated into newly synthesized proteins, eventually completely replacing the cells’ proteins, such that labeling efficiency is near 100%. The cell populations are then mixed together and digested for MS analysis to determine differential protein abundances.

16.4.1 Setting up a Database

Before running the walkthrough data, you need to set up a database. So that this can be a quick process, we have provided you with a sample fasta database called “SampleDB.fasta” in your PEAKS program folder (C:\PEAKS Studio 5.1\Data).
Click on the configuration toolbar icon or select “Configuration” from the “Tools” menu. Select “Database” from the left hand side of the window. Under “Database Details” enter the following information:

```
FASTA format database: UniProtKB/Swiss-Prot
```

Basic Options

- **Database name:** Sample DB
- **Path:** C:\Peaks Studio 5.1\Data\SampleDB.Fasta

You do not need to change any of the other information listed. Click the “Add/Update” button and then click “OK”.

### 16.4.2 Creating a project

Click on the “Create new project” icon or select “New project” from the “File” menu. Give a name to your project, for example “SILAC Sample”. Click Add data files. Locate “SILACSample.mzxml” from C:\PeaksStudio5.1\Data.

With the file highlighted, select, FT-trap from the “Instrument Type” drop-down list.

Click “OK”. Double click on the SILAC sample file. Your “Main Processing Screen” should look something like this:

For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once. You will need to wait until the file has loaded (i.e. the file node looks like this: 🌿).

See section 16.2 3D View to enable 3D view.
16.4.3 Setting up the Workflow Parameters

Click on the workflow button “Workflow”. The following window will appear:

Click “Select Data”. The following window will open:

Select the SILACSample.mzXML file and then click “Add to Right”. The samples should now appear in the “Selected Data” panel as shown below:
Click “OK”.

The workflow window should now display “finished” beside the “Selected Data” button.

Check the 2nd box and then click the “Refine data” button. Enter the following parameters:

![Workflow window with Refine data button highlighted]

Click “OK”.

Check the 3rd box and then click the “de novo” button. Enter the following parameters:

![Workflow window with de novo button highlighted]

Enter the PTMs as shown below by selecting “Set PTM”:

![PTM window with selected PTMs]

Click “OK”.

![PTM window with OK button highlighted]
Check the 4th box and then click the “PEAKS Search” button. Enter the following parameters:

Enter the PTMs as shown below by selecting “Set PTM”:
Check the 5th box and then click the “Quantification” button. Select “Label at the MS level” from the left hand panel and enter the following parameters:

![Workflow Configuration](image)

**Note:** To add information into the “Label Options” panel, click the “Add Label” button to add a new sample.

Click “OK” and then click “Start” in the workflow configuration panel. The following window will open:

![Workflow Summary](image)

Click “Start Jobs”.

124
16.4.4 SILAC Results

Once completed, the protein quantification result will be displayed in the PEAKS Protein ID node “.node”. Double click on this node the “Peptide View” results will appear by default:

Click on the “Peptide Details” tab to see where the selected peptide matches the protein highlighted in blue:
PEAKS provides the 3D View of each peptide feature for visual validation. Select the “3D View” tab. The panel along the bottom allows you to narrow in on the peptides that you would like to examine. You can specify a particular scan number range, m/z range or intensity range. Click the “Apply” button to change the 3D View to your specified values.

Select the “Protein View” tab. The SILAC quantification results are listed as a “Ratio Heavy: Light” and “Standard Deviation Heavy: Light”.

Note that the top protein result is Human Filamin A, with a score of 98.89%. The ratio of Heavy: Light is highlighted for each protein in the red box below. For example, the highest ranked protein, Human Filamin-A has a ratio of 1.2 and a standard deviation of 0.13.

A ratio of -1 means that while a reporter ion was found for the selected peptide, a ratio could not be calculated for the protein as the peptide did not meet the required score criteria (in this case we specified that the peptide score had to be greater than 0.6). A blank box in the ratio column means that a reporter ion for the selected peptide was not found.
There are four peptide features identified and calculated for quantification of Human Filamin-A. Two other peptides (Spectrum 40 and 49) were used to identify the protein but were not identified as features.

16.5 Label Free Quantification

Label Free quantification relies on the changes in analyte signals directly reflecting their concentrations in one sample relative to another. This technology employs overall spectral intensity normalization by interpreting signals of molecules that do not change concentration from sample to sample. By comparing two or more spectra, PEAKS can determine the constant intensity ratio between the unchanging analytes forms the basis for identifying the non-changing concentrations, making spiking unnecessary.

Below are instructions to take you through a label free dataset. Please be aware that the data files are fairly large and thus the computing time will be increased compared to the other walkthrough datasets explained in this manual.

16.5.1 Setting up a Database

Before running the walkthrough data, you need to set up a database. So that this can be a quick process, we have provided you with a sample fasta database called “SampleDB.fasta” in your PEAKS program folder (C:\PEAKS Studio 5.1\Data).

Click on the configuration toolbar icon or select “Configuration” from the “Tools” menu. Select “Database” from the left hand side of the window. Under “Database Details” enter the following information:
16.5.2 Creating a Project

Click on the “Create new project” icon or select “New project” from the “File” menu. Give a name to your project, for example “Label free walkthrough”. Click Add data files. Locate LFQSample1.mzXML. Then click “Add Sample” and Sample 2 will appear. Click “Add data files” to add “LFQSample2.mzXML” to Sample 2.

Note: For label free quantification to function in PEAKS, you need to have at least 1 sample with at least 1 file/fraction in each sample.

With one of the raw files highlighted, select FT-trap from the “Instrument Type” drop-down. Then click “whole project” to apply these parameters to the rest of the project.

Click “Ok”.

You do not need to change any of the other information listed. Click the “Add/Update” button and then click “OK”.

16.5.2 Creating a Project

Click on the “Create new project” icon or select “New project” from the “File” menu. Give a name to your project, for example “Label free walkthrough”. Click Add data files. Locate LFQSample1.mzXML. Then click “Add Sample” and Sample 2 will appear. Click “Add data files” to add “LFQSample2.mzXML” to Sample 2.

Note: For label free quantification to function in PEAKS, you need to have at least 1 sample with at least 1 file/fraction in each sample.

With one of the raw files highlighted, select FT-trap from the “Instrument Type” drop-down. Then click “whole project” to apply these parameters to the rest of the project.

Click “Ok”.
For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once. You will need to wait until the files have loaded (i.e. the file node looks like this: 🌟).

See section 16.2 3D View to enable 3D view.

16.5.3 Setting up the Workflow Parameters

Click on the workflow button “_workflow”. The following window will appear:

Click “Select Data”. The following window will open:

Click “All Samples” and then “Add to Right”. The samples should now appear in the “Selected Data” panel as shown:

Click “OK”.

129
The workflow window should now display “finished” beside the “Selected Data” button.

Check the 2nd box and then click the “Refine data” button. Enter the following parameters:

Click “OK”.

Check the 3rd box and then click the “De novo” button. Enter the following parameters:

Enter the PTMs as shown below by selecting “Set PTM”:

Click “OK”.
Check the 4th box and then click the “PEAKS Search” button. Enter the following parameters:

Enter the PTMs as shown below by selecting “Set PTM”:

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131
Check the 5th box and then click the “Quantification” button. Select “Label Free” from the left hand panel and enter the following parameters:

- Mass Error Tolerance: 0.2
- Peptide Score: Min. 0.5, Max. 1.0
- Protein Score: Min. 0.5, Max. 1.0

Click “OK” and then click “Start” in the workflow configuration panel. The following window will open:

Click “Start Jobs”.

16.5.4 Label Free Results

Once the workflow analysis is complete, you should see the following in your “Project View” panel:

Double click on the label free node. The identified proteins will appear in the top panel, with homologous proteins clustered together. The ratio of Sample 1: Sample 2 appears in the “Ratio” column and the standard deviation of Sample 1: Sample 2 appears in the “SD” column.
In the example below the standard deviation is 0 as there is only one supporting peptide for the protein shown.

The supporting peptide is shown under the “Peptides” tab. The retention time is shown for the specific peptide as well as the peptide ratio from Sample 1: Sample 2.

Click on the “+” beside the “Outlier” folder to see the peptides that were not included in the ratio.

To see which peptides were used to identify the protein during the PEAKS protein ID search, select the “Coverage” tab. The entire sequence of the protein is shown and the matching peptides are highlighted in blue. In this example the total matched part accounts for 9.392% of the protein. This information can be found in the “Coverage” column in the “Protein View” panel.

The features chart will appear by default in the bottom panel:

To see exactly where the selected supporting peptide corresponds to the protein sequence, select the “Peptide detail” tab. The entire sequence of the protein is shown and the selected supporting peptide is highlighted in blue.
Clicking on the “Quantification” tab will display a 2D view of the features. Move your cursor around the map to display the m/z ratio and retention time.

Right click your mouse and select “Show features” to display a 2D heat map. When viewing the heat map in color, red represents high intensity and yellow represents low intensity. The grayscale heat map displays high intensity in black while white represents low intensity.

Click on the “3D View” tab to display a 3D View of the peptide features for sample 1 and sample 2. Intensity is displayed on the y-axis, m/z on the x-axis and retention time on the z-axis. Click on the image and move the cursor to rotate.
17. References

de novo

SPIDER

Quantification
18. Appendix

18.1 Terminology and Abbreviations Glossary

**a-ions**: an N-terminal fragment holding at least one charge. This is a fragment of the peptide derived from b-ions. The a-ion’s mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, minus the mass of carbon monoxide.

**b-ions**: an N-terminal fragment holding at least one charge. This is a prefix fragment of the peptide. The b-ion’s mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues.

**BSI (Bioinformatics Solutions Inc.)**: The makers of PEAKS and other fine bioinformatics software.

**c-ions**: an N-terminal fragment holding at least one charge. This is a prefix fragment of the peptide. The c-ion’s mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, plus the mass of ammonia.

**Deconvolution**: rearrangement of the spectrum to show each monoisotopic peak as if it were singly charged. Thus, to reposition them on the scale, PEAKS multiplies the $m/z$ of ion’s that were doubly charged by two minus the mass of 1 H. Note that the deconvolved scale PEAKS shows is ‘at +1.’

**Fixed modification**: selecting a post-translational modification as a fixed modification tells PEAKS that this modification is applied to all occurrences of the residue(s) on which the PTM can occur.

**Enzyme**: Biomolecules that catalyze chemical reactions, including the digestion of proteins.

**ESI (Electrospray Ionization)**: A method for ionizing a sample into the mass spectrometer.

**$m/z$**: mass to charge ratio.

**MALDI (Matrix-Assisted Laser Desorption/Ionization)**: A method for ionizing a sample into the mass spectrometer. This has a characteristic effect of producing singly charged ions.

**Mass accuracy**: this refers to the accuracy of data obtained from a given mass spectrometer. On a spectrum, how close the peaks are to the actual masses of the ions they represent.
**PTM (Post-Translational Modification):** A newly translated protein may differ from its final form as a result of processing by various enzymes in the cellular environment. This change is referred to as a post-translational modification. Since PTMs change the mass of residues, it must be accounted for when sequencing peptides by mass spectrometry.

**Built-in PTM:** PEAKS comes equipped with a library of possible post-translational modifications. These can be incorporated into a *de novo* analysis at the click of a button.

**Customized PTM:** If the post-translational modification you are looking for is not in the PEAKS PTM set, you may create our own entry, or modify an existing one. This will appear as a customized PTM in the set.

**PTM library:** A listing of all possible (built-in and custom entered) post-translational modifications that PEAKS can use as a part of its analysis.

**Residue:** as used in this manual, a residue refers to what remains of an amino acid once it has become part of a peptide, or peptide fragment. In this manual, residues are referred to by their original amino acid names.

**Resolution:** refers to the resolving power of an instrument. On a spectrum, this is reflected by how close together two peaks can be and still be resolved.

**Variable modification:** selecting a post-translational modification as a variable modification tells PEAKS that this modification may or may not be applied to the residue(s) on which the PTM can occur.

**x-ions:** a C terminal fragment holding at least one charge. The x-ion’s mass will be the sum of the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of carbon monoxide.

**y-ions:** a C terminal fragment holding at least one charge. The y-ion’s mass will be the sum of the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of 2 H.

**z-ions:** a C terminal fragment holding at least one charge. The z-ion’s mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, after subtracting the mass of ammonia.

### 18.2 Toolbars

#### 18.2.1 Main Window Toolbar

- **New Project:** Clicking the New Project button will allow users to create a brand new project, offering organization to any study.

- **Add Data:** This allows you to add additional data to any existing project.
Open Recent Project: The quickest method to recalling an existing project. Users are instantly directed to a selection of recently created, modified or viewed PEAKS projects.

Close Project: When a project is complete or a user temporarily no longer is working on a particular project, users can click this icon to remove it from their Project View.

Save As: The project method of PEAKS allows for all results to be automatically constantly saved. However, if a user wishes to save a particular application under a new heading this is the most convenient and appropriate way to do so. Just click the icon and enter the new title in the field provided. The file will be saved in the ANZ format. Press this after selecting a data file in the Peptide Data Frame.

Print: Whether a user desires to print their ms spectrum views to complement a publication or print matrices, the print feature offers a straightforward connection to any printer configured to the user’s computer.

Export: Easily export the spectrum view, ion table, or a picture (bmp, gif, or jpg format) with ions, masses, PEAKS and peptides marked.

Exit: To exit from the PEAKS software safely, select this icon or press on the keyboard the ‘Control’ key simultaneously with the letter ‘Q’.

Data Refine: Merge scans of the same peptide, remove noise spectra, preprocess within each MS/MS spectrum and recover peptide charge state. The data refinement options dialogue will allow us to choose and to set parameters for each of these refinement tools.

de novo: Perform auto de novo for a selected data file, spectrum or project. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. An auto de novo options dialogue will allow users to set parameters before beginning.

PEAKS Search: Perform protein identification on a selected data project. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. A protein identification options dialogue will allow users to set parameters.

SPIDER Search: Peptide homologue search tool.

PTM Finder: A PTM finder search can be performed on any PEAKS Protein ID and allows the user to be able to identify more PTMs in less time.

inChorus Search: inChorus, a meta protein identification tool, can be used to compare and validate results, calling upon such search engines as Mascot, OMSSA, PEAKS, Sequest and X!Tandem, as well as SPIDER. Click this icon to set a task to have inChorus perform a user’s analysis easily.
Quantification: Where users require abundance ratio results, PEAKS Q provides insight. Click here to analyze results from ICAT, iTRAQ, Label Free, SILAC, N-terminal and User Defined Labeling techniques.

Workflow: A batch process, where users can analyze several samples at once by performing data refine, *de novo* sequencing, protein ID and quantification.

Configuration: Set up enzymes, post translational modifications, databases, instruments and parameters.

Preferences: Organize general properties such as directories, search engines and ion editing capabilities.

Mass Calculator: The Mass Calculator is a simple tool to help us determine the molecular weight of a peptide. Clicking this icon will make the mass calculator appear.

### 18.2.2 Project View

The Project View allows optimal organization and greater control when managing multiple files at once. As different projects may be active at once, it is important to understand the different categories and levels presented by PEAKS.

- **Tree Root**
- **Project Node**
- **Filter Result**
- **Sample Node**
- **File Node (when file is loading)**
- **File Node (when file is loaded)**

Search Engines: Within the Project View, these icons are used to represent which method/search engine a particular file was interpreted by.

- **M** Mascot
- **M** OMSSA
- **S** Sequest
- **X!** X!Tandem

### 18.2.3 Main Processing Window Toolbar

- **Profile Mode**
- **Peak Mode**
- **Return to original size**
- **Zoom X-axis**
18.3 Mass Calculator

Click the Mass Calculator icon on the toolbar or choose Mass Calculator from the Tools menu.

The following window will appear:

In order to use the mass calculator input the peptide sequence or enter the mass in Daltons. Indicate if the sequence contains b- or y ions or if your search is non-specific.

For example select “b-ion”, input the sequence ACDR and click the “Calculate” button. You should see the following:

You can change the charge and use the arrow on the right to calculate the precursor mass or use the arrow on the left to calculate the peptide mass.

Or for another example, change the margin of error to 1 Da, input a mass of 146 Da and click the “Calculate” button. In the sequences box you should see the following predicted sequences:

Use the “Clear” button to start again.
18.3.1 Advanced Options
Clicking on the “Advanced” button will display the following:

You will now be able to add various modifications to your amino acid sequence. For example, enter A, M, then select “Oxidation on M” from the PTM list and click the “PTM” button. Then click the “Calculate button. You should see the following:

To view additional PTMs from the unimod list, select the “unimod” box. You can delete a PTM using the “Delete PTM” button. You can also edit or create a new PTM using the “Edit PTM” or “New PTM” buttons, respectively.

You can also use the advanced options menu to add a water or proton to your sequence. The “Back” button will remove the last amino acid or modification that you have added to your sequence or click on the “Clear” button to start again.
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